Mechanism of vasopressin natriuresis in the dog: role of vasopressin receptors and prostaglandins

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Evidence has accumulated over the years that antidiuretic hormone [arginine vasopressin (AVP)], in addition to its principal action promoting water reabsorption in the collecting system of the nephron, can induce natriuresis in the dog (5, 8, 12, 16, 20, 30) and rat (2, 3, 18, 22, 23).

The mechanism of AVP natriuresis is unclear. Early studies suggested that the natriuretic effect is mediated by some humoral natriuretic substance (17, 21), presumably prostaglandins (PGs) (17). A decade ago, Bie and co-workers (5) showed that natriuresis occurred during infusions of AVP that raised the AVP plasma level to within the physiological range and that the response to deamino-[Cys1,D-Arg8]vasopressin (dDAVP), a specific agonist of vasopressin V2 receptor, did not include a natriuretic effect. Because PGs are natriuretic and AVP stimulates their intrarenal synthesis via V1 receptors (reviewed in Refs. 11 and 25), the principal renal PG, PGE2, has emerged as a possible mediator of AVP natriuresis.

The present study of the effect of physiological amounts of vasopressin in conscious dogs was designed to explore simultaneously a broad spectrum of factors that could contribute to natriuresis. Experiments were conducted under conditions of sustained servocontrolled hydration, and this protocol eliminated the possibility of natriuresis secondary to a progressing volume expansion or dissipation of medullary hypertonicity. The dose of vasopressin was fixed at a low level so as not to influence mean arterial blood pressure (BP) or glomerular filtration rate (GFR). Administration of dDAVP was used as a means to stimulate vasopressin V2 receptors without V1 receptor activation. Measurement of PGE2 excretion in different experimental series was included to expose the role of PG synthesis in the response to AVP.

MATERIAL AND METHODS

The study was designed to examine the action of approximately equipotent antidiuretic doses of AVP and the specific agonist of V2 receptors, dDAVP, under conditions of near complete suppression of endogenous secretion of AVP by water loading and constant body fluid volume. Vasopressin infusion was performed with or without blockade of PG synthesis with indomethacin (Indo). To avoid any major changes in the corticomedullary solute gradient, the infusion of antidiuretic peptides was started just before water loading.

The experiments were performed on seven conscious female beagle dogs weighing 9.5–13 kg and maintained on a fixed diet of commercial dog food (Febo Professional, Euskirchen, Germany) that provided a daily intake of sodium of ~6 mmol/kg body wt; free access to tap water was allowed.

Experimental procedures. In all animals, both common carotid arteries had been placed in skin loops while animals were under general anesthesia, with the use of sterile surgical technique. The dogs were trained to accept catheterization and to rest quietly for several hours supported in the upright position by a canvas sling. Each dog was used in each of four experimental protocols (see below) at intervals of >1 wk.

On the morning before each experiment, sterile catheters (Intracath, Deseret, UT) were placed in the external jugular and the saphenous veins and used for drug infusions and venous blood sampling, respectively. A short catheter placed in an exteriorized carotid artery allowed for measurement of arterial pressure using a Statham P50 transducer and for sampling of arterial blood. Heart rate was determined from the electrocardiogram. A modified indwelling Foley catheter was inserted into the urinary bladder.

Immediately after instrumentation, a constant infusion of antidiuretic peptide (AVP or dDAVP) or vehicle was initiated and continued throughout the experiment. To measure the GFR, a bolus of inulin or creatinine was injected (8 or ~14
mg/kg, respectively, in 7.4 ml of isotonic saline), followed by a continuous infusion that delivered 9.6 mg·kg⁻¹·h⁻¹ of inulin or ~14 mg·kg⁻¹·h⁻¹ of creatinine in a volume of 7.2 ml/h throughout the experiment.

At the time of bolus injection, the dogs were given a load of 20 ml water/kg body wt via a gastric tube. The state of overhydration was kept constant throughout the experiment by use of a servo system that maintained constant body weight (~0.2%) irrespective of the diuresis by delivering intravenously, at the appropriate rate, a hypotonic solution containing 40 mM glucose and 25 mM urea (4).

Plasma AVP levels and the variables characterizing excretion of water and solutes as well as urine concentration were measured for 150 min (5 30-min periods) beginning from the time point ~90 min after water loading and the start of AVP or dDAVP infusion. All the parameters leveled off during the last 30-min period representing a steady-state situation. Along with urine collections, venous blood samples were withdrawn for determination of plasma osmolality, Na⁺, K⁺, Li⁺, and inulin or creatinine. During antidiuresis, the bladder catheter was flushed two times with 10 ml of distilled water and then with 20 ml of air at the end of each period. Arterial blood samples for plasma and atrial natriuretic peptide (ANP) determination were drawn from the carotid artery at 30, 60, 120, and 150 min and were immediately centrifuged at 4°C. Plasma was stored at ~18°C until analysis.

The following series of experiments was performed in seven dogs: 1) control, i.e., a state of water diuresis (vehicle infusion at a rate of 0.75 ml·kg⁻¹·h⁻¹), 2) antidiuresis by AVP (50 pg·kg⁻¹·min⁻¹), 3) antidiuresis by dDAVP (4 pg·kg⁻¹·min⁻¹), and 4) antidiuresis (AVP, 50 pg·kg⁻¹·min⁻¹) combined with inhibition of PG synthesis (intravenous bolus injection of Indo, 4 mg/kg, given after the first urine collection period).

Analyses and statistics. Plasma and urine samples were analyzed for creatinine using the method of Bonsnes and Taussky (6) or for inulin using a chemical method based on fructose determination after acid hydrolysis of inulin (19). Plasma and urine Na⁺ and K⁺ concentrations were determined using a flame photometer (IL 343; Instrumentation Laboratory, Lexington, MA), osmolalities were determined by a freezing-point osmometer (Osmomat, Gonotec, Berlin, Germany), and plasma and urinary (endogenous) Li⁺ concentrations were determined by an atomic absorption spectrophotometer (Perkin-Elmer 5100 PC, Norwalk, CT) provided with a HGA furnace and an AS60 autosampler and using pyrocatechol graphite tubes and argon as a purge gas. Renal clearances of all substances were calculated from the standard formulas. The fractional proximal Na⁺ reabsorption was calculated as (GFR·Cᵢₒm·Pᵢₒm)/(GFR·Pᵢₙₑₒₐ), where Cᵢₒm is the clearance of endogenous lithium, Pᵢₒm is plasma sodium concentration, and GFR·Pᵢₙₑₒₐ is filtered sodium load.

For hormone determination, plasma samples were initially extracted on silica columns (Sep-Pak C₁₈, Waters, Milford, MA), and subsequently hormones were determined by RIA. AVP was determined by RIA as described by Sakurai et al. (28), with minor modifications (15). The recovery of unlabeled vasopressin was 83%; no correction was made for the loss during extraction. The detection limit was ~0.09 pg/ml. Because more than one-half of the samples contained undetectable amounts of vasopressin, they were set at the limit of detection to enable statistical analysis. This reduced the scatter but falsely increased the mean value, which would have been lower with an ideal assay. The RIA for ANP was performed by the method of Schütten and co-workers (29). Urine PGE₂ was measured by RIA as described by Christensen and Leyssac (10) and evaluated by Christensen et al. (9), with minor modifications.

The data are presented as means ± SE. Differences in mean values between series were evaluated by one-way ANOVA. In case of variance inhomogeneity, as indicated by Bartlett’s test, the data were transformed logarithmically before ANOVA (31). When F values indicated significance, differences were identified by a sequential variant of the studentized range method (Newman-Keuls test).

RESULTS

Plasma AVP levels and the associated values of water and solute excretion and urine concentration recorded at the end experiments, i.e., after an almost 4-h exposure to AVP or dDAVP, are shown in Fig. 1. Controlled sustained hydration (2% body wt) in the vehicle series resulted in extremely low plasma AVP (mean 0.12 pg/ml) and excretion of a large volume of urine (mean urine osmolality (Uₒₛₘ) 53 mosmol/kg H₂O). When plasma AVP levels under identical hydration in
the same dogs were maintained at a mean of 2.3 pg/ml by exogenous hormone infusion (AVP series), persistent antidiuresis was observed, with appreciable free water reabsorption and U\text{osm} exceeding 900 mosmol/kg (Fig. 1). Remarkably, mean osmolar clearance (C\text{osm}) was the same as in the water diuresis series (Fig. 1), indicating that the AVP-mediated antidiuresis was solely dependent on an increase in water reabsorption ("pure" antidiuresis). It will be shown below that C\text{osm} was preserved owing to an increase in sodium excretion (UNaV). U\text{osm} was highest when PG synthesis was inhibited in animals infused with AVP (AVP + Indo), evidently due to a large concentration in the urine of nonelectrolytes (urea). As expected, plasma AVP was very low in animals given dDAVP, whereas in the AVP + Indo series, it was similar to the series in which AVP was given alone.

Figure 2 collects data for UNaV and variables that usually determine its magnitude, such as arterial BP and GFR. To suitably relate natriuresis to the excretion of total solutes (U\text{osm}V), data for excretion of Na\textsuperscript{+} (UNaV) together with its attendant monovalent anion (U\textsubscript{2Na}V) are given rather than UNaV. In hydrated dogs given AVP alone, U\textsubscript{2Na}V was several times higher than in the three other groups. In this series, sodium salts constituted >40% of the excreted solutes (U\text{osm}V); the increment in U\textsubscript{2Na}V can be construed as a factor preventing a decrease in U\text{osm}V or C\text{osm}. Indeed, such a decrease did occur in the dDAVP and AVP + Indo series in which UNaV was similar to that observed during water diuresis (Figs. 1 and 2). In the AVP series, Na\textsuperscript{+} salts were the main ionic component of the urine, in contrast to the dDAVP series in which the contribution of sodium and potassium was similar (see Fig. 1, inset). A comparison of urine composition and flow between these two antidiuretic series shows that UNaV was 5.1-fold higher with AVP than with dDAVP infusion, due to a 2.7-fold higher sodium concentration (UNa) and only a 1.9-fold higher urine flow (V). In all four experimental series, BP and GFR, potential determinants of UNaV, were similar (Fig. 2). The similarity of proximal reabsorption (estimated from Li\textsuperscript{+} clearance) suggested no differences in the fluid outflow from the proximal tubule (data not shown).

PG excretion was remarkably high during water diuresis (Fig. 3), but because large variations in V invalidate the rate of excretion of PGs as an index of their intrarenal synthesis (16, 20, 32), the value cannot be compared with those measured in the three other series. An extremely low PG excretion in animals given Indo confirms the assumption that effective inhibition of PG synthesis was obtained by Indo. Mean PG excretion was highest in the AVP series in which major natriuresis was observed. It became gradually lower

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**Fig. 2.** Solute excretion (A), glomerular filtration rate (GFR, B), and blood pressure (MABP, C) during steady states after water loading without and with concomitant infusion of vasopressin or dDAVP (see MATERIAL AND METHODS). A: total solute excretion (U\text{osm}V, whole bar length) and Na\textsuperscript{+} anion excretion (U\textsubscript{2Na}V, hatched portion of bar) in 4 experimental series. *Significantly different from AVP series (P < 0.05).
per se from the possible influences of overhydration and extracellular volume expansion. Superimposition of these factors may have engendered controversies regarding specificity and interpretation of the AVP-mediated natriuresis in many past studies. To avoid this, the degree of hydration applied was modest (2% body wt) and yet sufficient to suppress endogenous AVP secretion, as confirmed by determination of plasma concentration. Second, owing to the servocontrol of the body weight of the animals throughout the experiments, a steady state of body fluid volume was achieved and maintained in all experimental series. Third, the protocol used here, in which AVP was administered from the very beginning ("hormone first") rather than during an ongoing water diuresis ("hydration first"), was not associated with a partial dissipation of the corticomedullary solute gradient, which in itself could induce natriuresis (see below).

The AVP natriuresis was apparent at the beginning of urine collection i.e., 90 min after the start of hormone infusion, and tended to increase modestly until the end of experiment (240 min of AVP infusion). These values are in accord with those obtained by Cowley et al. (12), who observed AVP natriuresis in dogs with servocontrolled body fluid volume. The rate of hormone infusion was sevenfold higher and the estimated plasma concentration was about fivefold higher than measured in this study. It should be emphasized that with their chronic infusion experiments, AVP natriuresis disappeared after 1 day. Obviously, the present investigation of the mechanism of AVP natriuresis concerns the early effect only.

The state of water diuresis subsequent to hydration ("water diuresis" series) was the necessary time control for the principal AVP series. However, such a control cannot be regarded as optimal. A massive flow along the collecting system of the tubular fluid (with presumably low UNa i.e., unfavorable gradient for its reabsorption) may result in natriuresis. The dDAVP series, included with the aim of avoiding stimulation of vasopressin V1 receptors, provided a more adequate "antidiuretic" control for AVP administration. Remarkably, specific stimu-
igation of only V₂ receptors failed to induce natriuresis, a finding highlighted by the fact that the intratubular hydraulic conditions in this series were presumably very similar to those of the AVP series. It appears therefore that stimulation of non-V₂ receptors is a prerequisite for, although not necessarily the only cause of, AVP natriuresis.

Activation of vasopressin receptors could lead to natriuresis via a number of mechanisms. Because the dose of AVP applied was subpressor and mean arterial BP was similar in all experimental series, a pressure natriuresis can safely be excluded. AVP has occasionally been reported to increase GFR (13), possibly by constriction of the efferent arteriole of the glomerulus, and in one study the natriuresis observed after prolonged infusions of AVP was associated with increasing GFR (30). However, in the present experiments the modest dose of AVP did not measurably change GFR.

It has been found that AVP may stimulate the release of the atrial natriuretic factor (ANP), probably via V₁ receptors (24). However, under our experimental conditions no such stimulation was observed. Indeed, plasma ANP tended to be lower in the AVP series than, for instance, in the dDAVP series. This seems to exclude the possibility that ANP could act as a mediator of AVP natriuresis.

Vasopressin infusion has been reported to inhibit the renin-angiotensin-aldosterone system (30). However, this effect was apparently due to body fluid expansion because it was not observed when the fluid volume was servocontrolled (12). Therefore, it appears unlikely that a vasopressin-mediated inhibition of the release of renin played a significant role in our experiments.

AVP is also a known stimulator of intrarenal biosynthesis of PG via V₁ receptors; a similar role of V₂ receptors is controversial (reviewed in detail in Ref. 11). The renal excretion of PGE₂, the major renal PG, is commonly regarded as a reliable index of the intrarenal PG synthesis under most experimental conditions. One notable exception is that when diuresis varies considerably, PG excretion increases with increasing V (16, 20, 32). It is very probable that relatively high PG excretion in our water diuresis series was related to the high V, which was 15–45 times higher than that seen in the three antidiuretic series.

When only antidiuretic conditions are compared, it is apparent that PGE₂ excretion was highest in the AVP series, compatible with stimulation of PG synthesis via vasopressin V₁ receptors. Within this series, PG excretion was highly correlated with U₁NV, more than with V. Lower PG excretion in the dDAVP series presumably reflected some baseline intrarenal synthesis. U₁NV was similarly low with the basal PG activity (dDAVP series) and during major inhibition of PG synthesis with Indo (AVP + Indo), suggesting no tonic PG-dependent natriuresis at a normal activity level.

Previous data documenting the relationship between U₁NV and PG were thoroughly discussed in recent reviews (11, 25), and the discussion may be used as a basis for a possible explanation of the AVP natriuresis observed in the present study. In hydrated dogs receiving AVP infusion, augmented synthesis of PG, especially of PGE₂, the major renal species, could increase U₁NV by a number of mechanisms. An obvious one would be a direct inhibition of sodium reabsorption in the cortical and medullary collecting duct, as reported from studies with isolated microperfused tubule segments. An inhibition by PG of AVP-dependent NaCl reabsorption in the medullary ascending limb of the loop of Henle, an action well documented in some species (albeit not in the dog), may have contributed to overall inhibition of NaCl reabsorption both directly and by reducing interstitial hypertonicity of the renal medulla. Partial dissipation of medullary hypertonicity after activation of PG biosynthesis could occur by other, perhaps more important, mechanisms. Earlier studies have indicated that most PGs (including PGE₂) and arachidonic acid increase glomerular juxtamedullary blood flow more than superficial cortical flow. Thus flow through the vasa recta could increase considerably and lead to a “washout” of medullary solutes. Still another mechanism for reduction of medullary hypertonicity would involve a documented reduction by PGs of collecting duct cell permeability to urea and limitation of urea back diffusion in this segment. A reduction of the corticomedullary osmolar gradient would impair tubular reabsorption of NaCl. Decreased tonicity of medullary tissue would reduce reabsorption of water from descending limbs of the loops of Henle, leading to increased delivery of fluid with a lower sodium concentration to more distal tubule sites.

Our data cannot distinguish among the various mechanisms potentially responsible for natriuresis. However, the observation in the AVP series that the increase in U₁NV was to a large extent due to an increase in U₁Na and to a less extent to an increase in urine volume speaks in favor of an important role of direct inhibition of tubular NaCl transport. Data of the literature suggest distal nephron segments as sites of transport inhibition, in accordance with our measurements suggesting the constancy of proximal reabsorption.

A possible PG mediation of AVP natriuresis in the dog was first suggested by Fejes-Toth and co-workers (17). Lote and co-workers (23) studied the role of PG in the rat and concluded that PGE₂ was not involved in the natriuresis. However, their data indicated that with an AVP infusion rate of 50 pmol·h⁻¹·kg body wt⁻¹, Indo significantly attenuated the observed increase in U₁NV. Important species differences may be expected: studies of microdissected cortical collecting ducts indicated that PGE₂ inhibited cAMP accumulation induced by AVP in the rabbit but not in the rat kidney (7). Others have reported very slight (3) or nonexistent (27) AVP natriuresis in the rat, and sodium retention was reported from some studies in humans (1, 26).

In summary, the present results indicate that in hydrated dogs physiological amounts of AVP induce natriuresis, at least within the first hours of exposure to the hormone. The natriuresis was specifically related to the peptide action and not to the consequence of
hydration or extracellular volume expansion per se. It was present in the absence of any increase in arterial BP, GFR, or plasma ANP activity. On the other hand, the data provide evidence that AVP natriuresis was mediated by PGs.

Perspectives

Vasopressin released in response to an increase in plasma osmolality helps to correct the primary abnormality mainly by promoting water reabsorption via its hydroosmotic effect on the collecting system. Another way of bringing body fluid tonicity back toward normal would be disposal of solutes. Vasopressin natriuresis could serve as such an auxiliary mechanism; its advantage is that it is rapid in onset. Its short duration (from several hours to 1 day) may not be a homeostatic disadvantage, because persisting antidiuresis leads to extracellular volume expansion and ultimately elevation of BP, both situations inducing natriuresis by mechanisms not directly related to the hormone.

Despite the present evidence suggesting the role of vasopressin V₁ receptors and intrarenal PG, the exact mechanism of AVP natriuresis has not been satisfactorily elucidated. Application of specific agonists and antagonists of vasopressin V₁ and V₂ receptors, in addition to dDAVP, could help accomplish this goal. This would help to clarify the degree of overlap between the water-retaining control system in which vasopressin is the main effector and the sodium-retaining control systems dominated by renin, angiotensin, and aldosterone.

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