Mechanisms of Escape from Desmopressin in the Rat

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SUMMARY. The mechanism(s) of renal escape from the hydro-osmotic effect of vasopressin is unknown. We therefore studied escape in conscious, unrestrained rats receiving continuous intravascular infusions of 1-deamino-8-arginine-vasopressin (desmopressin) and hypotonic fluid over 5 days. Escape from desmopressin started 8 hours after exposure and was characterized by a progressive increase in urine flow and decreases in urine osmolality and free water reabsorption. When positive water balance was prevented by matching the rate of infusion of hypotonic fluid to urine flow while maintaining the dose of desmopressin constant, escape did not occur. This suggested that water retention, rather than chronic exposure to desmopressin, mediated the escape. To elucidate the mechanism whereby water retention induces escape from desmopressin, urinary prostaglandin E$_2$ excretion was measured and found to be increased concomitant with the onset of escape. Prevention of this increase in urinary prostaglandin E$_2$ excretion with indomethacin resulted in additional water retention and a delay in the onset of escape. During the maintenance of escape, after significant water retention occurred, increases in mean arterial pressure, renal blood flow, and glomerular filtration rate were observed. Renal interstitial solute concentration remained constant through escape. Basal and vasopressin-stimulated collecting tubular and thick ascending limb adenylate cyclase did not differ when control and escape animals were compared. These results suggest that enhanced renal synthesis of prostaglandin E$_2$ facilitates the early phase of escape; later, water retention results in plasma volume expansion with increases in cardiac index, arterial pressure, renal blood flow, and glomerular filtration rate. These systemic and renal hemodynamic alterations may be important in maintaining escape from desmopressin.


MAINTENANCE of normal body water balance is important in regulation of plasma tonicity and body fluid spaces. The action of the posterior pituitary hormone vasopressin to increase water permeability of renal collecting tubular epithelium is the major mechanism controlling water loss. Although vasopressin exerts initially marked renal water retention, a significant decline in this hydro-osmotic effect occurs after chronic vasopressin exposure (Chan, 1971; 1973; Davis et al., 1954; Jaenike and Waterhouse, 1961; Kaehny et al., 1978; Leaf et al., 1953; Levinsky et al., 1959a; Smith et al., 1979; Stokes, 1981; Stormont, Waterhouse, 1961). This decrease in hydro-osmotic response has been termed "vasopressin escape" (Chan, 1973). Clarification of the mechanism whereby chronic vasopressin down-regulates its own action would lead to a greater understanding of renal water excretion. However, despite recognition of vasopressin escape more than 25 years ago (Davis et al., 1954), the mechanism responsible for this phenomena has not been elucidated.

We recently observed that the antidiuretic effect of vasopressin (1-deamino-8-arginine vasopressin, desmopressin) declines progressively over 5 days of administration to conscious rats (Gross and Anderson, 1982). The present experiments were designed to clarify pathogenetic factors involved in this diminished hydro-osmotic response. We first determined that water retention, rather than chronic exposure to desmopressin, was necessary for the decrease in renal water retention. Next, we assessed the role of prostaglandin E$_2$ and intrarenal factors such as glomerular filtration rate, renal hemodynamics, collecting duct environment, and adenylate cyclase in the pathogenesis of the diminution in desmopressin response.

Methods

Experiments were carried out in conscious, unrestrained male Sprague-Dawley rats weighing 320–400 g. Animal preparation and specific aspects of the experimental protocol have been described in detail (Gross and Anderson, 1982; Gross et al., 1982). Briefly, an 18-gauge plastic catheter (Angiocath, Deseret) was doubly ligated into the left carotid artery and then connected to a fluid-filled ruber catheter that was tunneled under the skin. The catheter was doubly ligated into the left carotid artery under pentobarbital anesthesia 48–72 hours prior to study. The catheter was tunneled under the skin over the cervical spine where the exit site was sealed with a rubber cap. Needle puncture of this cap provided vascular access for blood sampling, continuous intravascular infusions, and direct measurement of arterial pressure.

Basic Protocol

All studies were carried out in metabolic balance cages that allowed for separation of urine and feces (Holtage Comp.). All animals were conscious and unrestrained during the 5-day study period. Room temperature was maintained at 65°F and relative humidity at 30%. Most animals underwent continuous infusions of hypotonic fluid (0.22% NaCl) at 3.1 ml/hr over 5 days. Previous studies
demonstrated that this rate of infusion of 0.22% NaCl does not alter the hydro-osmotic effect of desmopressin (Gross and Anderson, 1982). Control animals (group 1) were infused only with 0.22% NaCl throughout the study interval. Experimental animals (groups 2, 3, and 4) also received desmopressin (Ferring Pharmaceuticals) at 0.112 ng/hr commencing 24 hours after the start of 0.22% NaCl. Desmopressin rather than arginine vasopressin (AVP) was utilized in the present studies because of greater ease in preparation, longer half-life, fewer vascular effects, and better stability in solution. Moreover, in contrast to desmopressin, escape from AVP is associated with significant natriuresis and negative sodium balance (Gross and Anderson, 1982).

All infused fluids were drawn into 35-ml plastic syrings and delivered at a constant rate by electric infusion pumps (Razel H-99, Razel Scientific Instruments) and renewed at 8-hour intervals. Animals had no access to fluid other than the fluid they received via intravenous infusions. Control and experimental animals were paired standard rat chow (Wayne Lab-Blox, Allied Mills) throughout the study. In all animals, urine was collected at 8-hour intervals throughout the study and analyzed for volume, osmolality (Advanced Instruments Osmometer), and glucose, ketones, and hemoglobin (Ames Dipstix, Ames Corp.). Free water reabsorption was calculated by standard techniques. Rats were weighed at 12 to 24-hour intervals. Blood (0.4 ml) was sampled from the intracardiac catheters at 12- to 24-hour intervals for analysis of sodium (IL Flame Photometer, Beckman). The magnitude of water retention in experimental animals was determined as described previously (Gross and Anderson, 1982). The role of water retention.

Role of Water Retention

These studies were undertaken to evaluate the respective roles of water retention vs. prolonged exposure to desmopressin per se in the escape phenomenon. In these studies, we compared the pattern of escape from desmopressin in three groups of animals. A control group (group 1, n = 21) received 0.22% NaCl at 3.1 ml/hr throughout the study. A second group (group 2, n = 21) received 0.22% NaCl at 3.1 ml/hr throughout the study with 0.112 ng/hr of desmopressin added after the first 24 hours of 0.22% NaCl. Data obtained in these two groups have been published previously (Gross and Anderson, 1982). In the third group (group 3, n = 8), progressive water retention was prevented by adjusting the rate of infusion of 0.22% NaCl to match urine flow on an 8-hour basis simultaneous with the start of desmopressin. Matching the rate of infusion of 0.22% NaCl to urine flow prevented a large positive water balance in these animals. The concentration of desmopressin in the infusions of these group three animals was also changed on an 8-hourly basis to maintain the rate of desmopressin infusion constant at 0.112 ng/hr.

Role of Prostaglandin E₂

These studies were undertaken to elucidate the mechanism whereby water retention results in escape from desmopressin. The role of prostaglandin E₂ in the escape phenomenon was assessed because previous experiments have demonstrated an effect of products of cyclo-oxygenase (most likely prostaglandin E₂) to antagonize the hydro-osmotic effect of vasopressin in the intact rat kidney (Berl et al., 1977; Lum et al., 1977). Since urinary excretion of prostaglandins reflects renal prostaglandin biosynthesis (Zambraski and Dunn, 1979), we first examined the relationship between escape from desmopressin and urinary prostaglandin excretion. In these studies, 8-hour urine samples were analyzed for prostaglandin E₂ in four groups of rats: (1) 12 group 1 control animals (receiving only 0.22% NaCl at 3.1 ml/hr); (2) 12 group 2 experimental animals (receiving 0.22% NaCl at 3.1 ml/hr with 112 ng/hr of desmopressin added after 24 hours of 0.22% NaCl); (3) 8 group 3 experimental animals (receiving 0.22% NaCl matched to urine flow and 0.112 ng/hr of desmopressin after 24 hours of 0.22% NaCl); and (4) 10 group 4 experimental rats (treated identical to group 2 rats except that indomethacin at 1.75 mg/kg per day was infused continuously starting concomitantly with desmopressin). The dose of indomethacin utilized was determined by a series of preliminary trial-and-error experiments which ascertained that 1.75 mg/kg per day prevented the increase in urinary prostaglandin E₂ excretion observed in group 2 animals, while not decreasing urinary prostaglandin E₂ excretion rates lower than those observed in group 1 animals. Indomethacin (Sigma Chemical Corp.) was dissolved in sodium carbonate and distilled water, and prepared fresh every 8 hours. Urine samples were collected in 300 μl of 40% sodium azide at room temperature and frozen at -60°C until analysis. Urinary prostaglandin E₂ concentration was determined by radioimmunoassay using the method of Dray et al. as described previously (Berl et al., 1980; Dray et al., 1975). Hourly prostaglandin E₂ excretion rates were calculated from urinary concentration of prostaglandin E₂ and urine flow rates during collection. Eight-hour samples were obtained before starting and at 8–16 hours (day 2 of the protocol), 40–48 hours (day 3), and 64–72 hours (day 4) after starting desmopressin.

To assess the physiological significance of the observed increase in urinary prostaglandin E₂ excretion during escape from desmopressin, we determined water balance in animals in which indomethacin prevented an increase in urine PGE₂ excretion. In these studies, the magnitude of net water retention, the pattern of escape, and the steady state serum sodium levels at escape were compared in 9 group 2 and 10 group 4 animals studied simultaneously.

Role of Intrarenal Factors

These studies were undertaken because of the well-documented effect of glomerular filtration rate (Berliner and Davidson, 1957; Gellai et al., 1979; Levinsky et al., 1959), renal blood flow (Abbrecht and Malvin, 1961), solute excretion rate (Anslow Wesson, 1955; Atherton et al., 1971; deWardener and DelGreco, 1955), and renal interstitial tonicity (Atherton et al., 1971; Chan, 1973, 1971) to alter the hydro-osmotic effect of vasopressin. In these studies, glomerular filtration rate was estimated from measurements of endogenous creatinine clearance. Endogenous creatinine clearances were used because insulin infusions facilitated escape from desmopressin. To validate the use of endogenous creatinine clearance in these studies, simultaneous insulin and creatinine clearances were measured in a separate group of experiments performed on 10 group 2 animals before and during desmopressin administration. In these studies, a highly significant correlation (r = 0.72, n = 20, P < 0.05) between insulin clearance and endogenous creatinine clearance was observed. The slope of the relationship between endogenous creatinine clearance and insulin clearance indicates that creatinine clearance is less than insulin clearance. For clearance determinations, urine was collected over 16

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hours and serum was obtained at the mid-point of the urine collections. Creatinine clearances were measured at 24- to 48-hour intervals in 8 group 1, 11 group 2, and 8 group 3 animals.

To ascertain the mechanism of the observed increase in creatinine clearance during escape from desmopressin, mean arterial pressure and renal blood flow were measured, and renal vascular resistance was calculated in 8 group 1 and 8 group 2 rats at the time a significant increase in creatinine clearance was observed. Arterial pressure was measured with Statham transducers (Statham Instruments, Inc.) following direct puncture of the intracarotid catheter. For measurement of renal blood flow, animals were lightly anesthetized with ether and a tapered PE 50 tubing was advanced retrograde through the right carotid artery until a pressure tracing indicated an intraventricular position of the catheter. A second catheter was then placed in the femoral artery for blood collection. The animals were then transferred to restraining cages and allowed to stabilize for 60 minutes. Subsequently, intracardiac position of the carotid catheter and patency of the femoral arterial catheter were again demonstrated. Microspheres (3 M Company) labeled with Sr and measuring 8.8 ± 0.5 μm in diameter were dissolved in normal saline and injected according to techniques described previously (Linas et al., 1980).

To evaluate a potential role for osmotic diuresis in the escape phenomenon, hourly osmolar excretion rates were calculated from mean hourly urine flow rates and mean urinary osmolalities. Osmolar excretion rates were determined from 8 hour urine samples obtained throughout the experimental protocol in 21 group 1, 19 group 2, 10 group 3, and 8 group 4 animals.

To determine if a decrease in renal interstitial tonicity could underlie the escape phenomenon, we measured inner medullary (papillary) electrolyte (sodium and potassium) and urea nitrogen content in 8 group 1, 8 group 2, and 8 group 4 animals. Inner medullary solute content was measured at the time of early escape (16 hours after start of desmopressin) and during a late period of escape (80 hours after start of desmopressin). In addition, inner medullary solute content was also measured in 8 group 1 animals 8 hours before time of start of desmopressin and in 8 group 2 animals at maximal antidiuresis (8 hours after start of desmopressin). Animals were killed by guillotine and the kidneys removed rapidly. Inner medulla was rapidly dissected free and weighed. One intact inner medulla was homogenized in 0.5 ml of deionized water for measurement of sodium and potassium (Perkins Elmer Atomic Absorption Spectrophotometer, model 295) and urea nitrogen content was calculated as described by Appelbaum et al. (1958).

Role of Biochemical Factors in Escape from DDAVP

To ascertain whether biochemical abnormalities occurred during escape from DDAVP, we measured adenylate cyclase activity and cAMP-phosphodiesterase activity in outer and inner renal medullary tissue. Measurements of these enzyme activities were undertaken in 7 control and 7 experimental animals during early escape and in 7 control and 8 experimental animals during late escape. In these experiments, rats were anesthetized by intraperitoneal pentobarbital (50 mg/100 g body weight). The kidneys were exposed by careful dissection and harvested.

The entire kidney was sliced coronally, just avoiding the midline, and the intact inner and outer medullary portion was removed in toto. The renal medulla was then dissected into outer and inner medullary portions and washed in a large volume of homogenizing medium of the following composition: 250 mM sucrose, 3 mM MgCl₂, 1 mM EDTA, and 5 mM Tris-HCl, at a pH of 7.4. Adenylate cyclase and phosphodiesterase activity was determined in these studies by methods described recently from our laboratory (Anderson et al., 1982; Kim et al., 1977).

Studies in Medullary Tubules

Since whole rat renal medullary tissue contains vasopressin responsive cAMP system components from renal tissue other than collecting tubule, adenylate cyclase activity and cAMP phosphodiesterase activity were measured in dissected medullary nephron segments. In these studies, collecting tubules and thick ascending limbs of Henle's loop dissected from outer medulla were utilized. Tubules were obtained from control animals (n = 10) and experimental animals (n = 5) at the time of early escape (16 hours after DDAVP).

Mediodissection of tubular segments was performed as described by Morel et al. (1976a, 1976b) and Jackson et al. (1980). Medullary collecting tubules (MCT) and thick ascending limbs of Henle's loop were microdissected under a stereomicroscope using thin needles. The isolated segments were transferred in a small volume of isotonic microdissection solution to a concave slide containing a drop of 0.05% bovine serum albumin. Tubules were quickly photographed, microdissection solution removed, and 0.5 μl of hypo-osmotic solution (0.25 mM EDTA, 1 mM MgCl₂, 0.1% bovine serum albumin, and 1 mM Tris-HCl, pH 7.4) added to the samples. The tubular segments in hydro-osmotic solution were frozen on dry ice and thawed on ice twice for adenylate cyclase assay and phosphodiesterase assays.

A determination of adenylate cyclase was performed as described by Morel et al. (1976a) with a slight modification. Reaction medium was added to the tubules of 5 μl of ATP regenerating medium containing 3.8 mM MgCl₂ and 20 mM creatine phosphate, 1 mg/ml creatinine phosphokinase, 0.25 mM cAMP, 0.25 mM EDTA, 20 mM creatinine phosphate, 1 mg/ml creatinine phosphokinase, 0.25 mM α-32P-ATP (4 to 6 × 10⁶ counts/min), and 100 mM Tris-HCl, pH 7.4. The slides were then incubated at 37°C for 30 minutes. The reaction was stopped by adding ice-cold 150 μl of stopping solution (3.3 mM ATP, 5 mM cAMP, 50 mM Tris-HCl, pH 7.6, and about 10,000 counts/min 32P-cAMP for recovery). This mixture was transferred to a 12 × 75 mm tube and washed with another 75 μl of stopping solution. Produced 32P-cAMP was separated by two-step elution, with Dowex-50 and alumina columns (Solomon, 1979). Adenylate cyclase activity was determined with synthetic arginine vasopressin (Calbiochem) at a concentration of 10⁻⁶ M.

Measurement of cAMP-phosphodiesterase was performed by a modified method of Wells et al. (1975) and Jackson et al. (1980). The activities were measured by final volume of 5 μl of reaction buffer containing 10 mM MgSO₄, 0.1 mM EDTA, 1 × 10⁻⁶ M 3H-cAMP (2 to 3 × 10⁵ counts/min), and 50 mM Tris, pH 8.0, and was incubated at 37°C for 20 minutes. The reaction was stopped by adding 50 μl of 5 mM acetic acid, and was transferred to a 12 × 75 mm tube. The slides were washed with 50 μl of 5 mM acetic acid and pooled. The tubes were immediately placed into a boiling water bath for 3 minutes and cooled to room temperature.
Results

Role of Water Retention

The rats appeared unaware of the infusions, and moved normally in their cages throughout the protocol. Glycosuria, ketonuria, and hemoglobinuria were not observed during the study. Observations on urine flow rate and urinary osmolality in groups 1 through 3 are depicted in Figures 1 and 2. In experimental group 2 animals, urine flow decreased and urinary osmolality increased in the first 8 hours following desmopressin. From 8 to 16 hours after desmopressin, a brisk increase in urine flow rate and a decrease in urinary osmolality occurred. However,
neither urine flow rate nor urinary osmolality returned to control levels until 64–80 hours after start of desmopressin (days 4 and 5 of the protocol). Free water reabsorption varied between 1.15 ± 0.1 and 1.00 ± 0.1 ml/hr in control animals throughout the protocol. Maximum free water reabsorption in experimental animals occurred within the 8-hour period following start of desmopressin (2.62 ± 0.2 ml/hr). Thereafter, a progressive decline in free water reabsorption was noted, and free water reabsorption was 1.70 ± 0.13 ml/hr at the end of the protocol in the experimental animals.

Desmopressin-treated animals retained an average of 30.9 ± 2.0 ml of water during the 5 days of infusion. To assess the role of this water retention in the escape phenomenon, we studied the pattern of escape in experimental animals (group 3) in which positive water balance was prevented (Figs. 1 and 2). As can be observed in Figures 1 and 2, if positive water balance was prevented, escape from desmopressin did not occur. In these group 3 animals, free water reabsorption remained between 2.5 and 3.0 ml/hr throughout the protocol. Although we attempted to maintain neutral water balance in these animals, a net gain of 8.0 ± 3.2 ml of water occurred. This resulted in a decrease in serum sodium from 143 ± 0.6 to 140 ± 0.8 mEq/liter (P < 0.05). Mean arterial pressure did not change significantly throughout the protocol (130 ± 6 start, 125 ± 5 mm Hg finish, NS).

Role of Prostaglandins E₂

Urinary excretion rates of prostaglandin E₂ in groups 1, 2, 3, and 4 animals are shown in Figure 3. Control animals (group 1) demonstrated relatively constant urinary prostaglandin E₂ excretion rates throughout the protocol. In contrast, urinary prostaglandin E₂ excretion rates increased significantly (P < 0.05) in experimental group 2 animals immediately after desmopressin. Thereafter, urinary prostaglandin E₂ excretion rates remained slightly but not significantly higher than in control group 1 animals. No increase in urinary prostaglandin E₂ excretion rates occurred in group 3 animals that received comparable amounts of desmopressin as group 2, but were without positive water balance due to matching of infusion rates of 0.22% NaCl to urine flow rates. Urinary prostaglandin E₂ excretion rates in animals receiving indomethacin in addition to desmopressin and 0.22% NaCl did not differ significantly from those in group 1 control animals.

The effect of indomethacin on water balance in animals receiving desmopressin and 0.22% NaCl (group 3) is shown in Figures 4 and 5. Indomethacin resulted in significantly enhanced and prolonged initial antidiuretic response to desmopressin (Fig. 4). Indomethacin also produced a significant increase in urine osmolality in the first three periods following desmopressin. Steady state serum sodium concentrations were also significantly lower in indomethacin-treated animals (Fig. 5). Net water retention 36 hours after start of desmopressin was 19.0 ± 4.6 in non-indomethacin-treated animals (group 2) and 34.0 ± 3.52 ml (P < 0.05) in indomethacin-treated animals (group 4). Thus, indomethacin increased the magnitude and duration of the antidiuresis after desmopressin and resulted in lower steady state plasma sodium concentrations. However, escape from desmopressin ultimately occurred even in the absence of an increase in urinary PGE₂ excretion.

Role of Intrarenal Factors

Sequential measurements of endogenous creatinine clearance in groups 1 through 3 animals are shown in Figure 6. Animals that did not escape from desmopressin (groups 1 and 3) demonstrated constant values for creatinine clearances throughout the protocol. In contrast, significant increases in creatinine clearance were observed during the later phase in animals that escaped from desmopressin (group 2). In addition, a significant increase in creatinine
clearance was observed during the later phase of escape in indomethacin-treated animals (group 4, 1 day after desmopressin, 0.40 ± 0.05; day 4 postdesmopressin, 0.54 ± 0.09 ml/min per 100 g body weight, \( P < 0.05 \)). These increases in creatinine clearance in animals that escaped from desmopressin occurred after the onset of escape.

To ascertain the mechanism of the observed increase in creatinine clearance during late escape, renal blood flow was measured at the time an increased creatinine clearance was measured. Renal blood flow was increased significantly when experimental group 2 rats were compared with control group 1 rats (8.30 ± 0.55 vs. 5.7 ± 0.29 ml/min per kg body weight, \( P < 0.02 \)). This increase in renal blood flow in experimental animals was due to both an increase in renal perfusion pressure (controls 110 ± 4.0, experimental 131 ± 5.0 mm Hg, \( P < 0.05 \)) and a decrease in renal vascular resistance (controls 19.3 ± 0.8, experimental 12.0 ± 0.6 mm Hg/min per kg body weight, \( P < 0.05 \)). As published previously, mean arterial pressure in experimental animals rose progressively throughout the protocol (Gross and Anderson, 1982).

Inner medullary solute concentration in groups 1, 2, and 4 animals are given in Figure 7. Shortly after the onset of escape from desmopressin, significantly higher inner medullary solute content was present in animals that escaped (group 2) than in control animals (group 1). During a later phase of escape, inner medullary solute content was comparable in all three groups. Thus, escape from desmopressin could not be attributed to a diminution in inner
medullary interstitial tonicity. Serial measurements of osmolar excretion in all groups (Table 1) revealed that escape occurred even when osmolar excretion did not increase (group 4).

Role of Biochemical Factors

In vitro studies of adenylyl cyclase activity in inner and outer medulla during early and late escape in control and experimental animals demonstrated comparable values for basal and stimulated adenylyl cyclase during both phases of escape (Table 2). cAMP phosphodiesterase activity was comparable in control and experimental outer medulla during early and late escape from DDAVP. In addition, cAMP phosphodiesterase activity in control and experimental inner medulla during early (248.4 ± 14.0 vs. 244.7 ± 12.4 fmol/µg protein per min, respectively, NS) and late escape (375.0 ± 21.8 vs. 373.9 ± 22.9 fmol/µg protein per min, respectively, NS) did not differ significantly.

In vitro measurements of adenylyl cyclase response to arginine vasopressin and NaF were undertaken in isolated tubular segments of outer medullary tissue and are in Table 3. Basal activities of adenylyl cyclase were comparable in both groups of animals. In addition, both groups of tubules responded to stimulation with either 10⁻⁶ M arginine vasopressin or 10⁻⁴ M sodium fluoride in a comparable fashion. cAMP phosphodiesterase activity in dissected tubules is in Table 4. No differences were observed.

Discussion

Although escape from the renal hydro-osmotic effect of vasopressin has been well documented (Chan, 1973, 1971; Davis et al., 1954; Jaenike and Waterhouse, 1961; Kaehny et al., 1978; Leaf et al., 1953; Levinsky et al., 1959a), the mechanism underlying this phenomenon has not been clarified. In the present experiments, intravascular infusions of hypotonic fluid and desmopressin resulted in a 13–14% net gain in total body water before water balance was restored (Gross and Anderson, 1982). The role of this positive water balance in mediating...
**Escape from Desmopressin**

Table 2: Adenylate Cyclase Activity* Response to Arginine Vasopressin and Sodium Fluoride in Inner and Outer Rat Medullary Tissue During Escape from DDAVP

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>10⁻⁴ AVP</th>
<th>NaF</th>
<th>X†</th>
</tr>
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<tbody>
<tr>
<td><strong>Early escape</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Outer medulla</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n = 7)</td>
<td>15.9 ± 1.6</td>
<td>43.1 ± 3.8</td>
<td>2.7 ± 0.5</td>
<td>73.8 ± 10.0</td>
</tr>
<tr>
<td>Experimental (n = 7)</td>
<td>22.1 ± 3.3</td>
<td>44.8 ± 4.3</td>
<td>2.0 ± 0.5</td>
<td>79.0 ± 7.8</td>
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<tr>
<td>Inner medulla</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n = 7)</td>
<td>29.6 ± 6.0</td>
<td>48.9 ± 10.3</td>
<td>1.7 ± 0.5</td>
<td>94.3 ± 11.5</td>
</tr>
<tr>
<td>Experimental (n = 7)</td>
<td>24.0 ± 3.3</td>
<td>48.9 ± 3.5</td>
<td>2.0 ± 0.6</td>
<td>113.9 ± 16.0</td>
</tr>
</tbody>
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Late escape

<table>
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<tr>
<th></th>
<th>Basal</th>
<th>10⁻⁴ AVP</th>
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<th>X†</th>
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<tbody>
<tr>
<td>Outer medulla</td>
<td></td>
<td></td>
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<tr>
<td>Control (n = 8)</td>
<td>18.8 ± 1.6</td>
<td>28.6 ± 2.6</td>
<td>1.5 ± 0.3</td>
<td>44.0 ± 3.5</td>
</tr>
<tr>
<td>Experimental (n = 7)</td>
<td>17.2 ± 1.3</td>
<td>29.6 ± 3.6</td>
<td>1.7 ± 0.4</td>
<td>45.2 ± 5.4</td>
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<tr>
<td>Inner medulla</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n = 8)</td>
<td>22.7 ± 1.8</td>
<td>37.0 ± 2.5</td>
<td>1.6 ± 0.5</td>
<td>53.2 ± 5.1</td>
</tr>
<tr>
<td>Experimental (n = 7)</td>
<td>19.9 ± 2.0</td>
<td>28.9 ± 3.2</td>
<td>1.5 ± 0.4</td>
<td>47.7 ± 7.4</td>
</tr>
</tbody>
</table>

* Expressed as pmol cAMP/min per mg protein.

escape from desmopressin was first examined. In these studies, marked positive water balance was prevented by matching the rate of infusion of hypotonic fluid with urine flow while the dose of desmopressin was maintained constant. It should be acknowledged, however, that the exact volume status of these volume matched animals was not ascertained. Although the measured positive water balance and stable levels of arterial pressure and creatine clearance suggest a euvolemic state, additional studies in this area appear warranted. When positive water balance was thus prevented, escape from desmopressin did not occur. In older studies, chronic administration of pitressin tannate in oil without hypotonic fluid did not impair the hydro-osmotic effect of pitressin (Chan, 1973; Levinsky et al., 1959b). In a preliminary communication, use of a sophisticated system to prevent positive water balance also prevented vasopressin escape (Cowley et al., 1983). Together, the present and previous studies suggest that chronic exposure to vasopressin per se is not sufficient to result in escape from this hormone.

There are several mechanisms whereby the combination of desmopressin and hypotonic fluid could result in a diminished hydro-osmotic response. For example, a failure of desmopressin to increase collecting duct water permeability would result in a decrease in renal water retention. With regard to this possibility, we undertook studies on prostaglandin E₂ (Gross et al., 1981; Stokes, 1981). These studies were performed for three reasons: (1) in vitro studies in toad urinary bladder and rabbit collecting tubule demonstrate that prostaglandin E inhibits the water permeability effect of vasopressin (Grantham and Orloff, 1968; Orloff et al., 1965); (2) prostaglandin E₂ appears to antagonize the hydro-osmotic effect of antidiuretic hormone in the in vivo rat kidney (Lum et al., 1977; Berl et al., 1977); and (3) vasopressin increases renal biosynthesis of prostaglandin E₂ in the rat (Dunn et al., 1978; Walker et al., 1978). In the present studies, an increase in urinary prostaglandin E₂ excretion rate was observed early following desmopressin. This increase in urinary prostaglandin E₂ excretion could not be attributed to desmopressin alone, since animals receiving comparable doses of desmopressin in which positive water balance was prevented by matching the infusion rate of hypotonic fluid to urine output did not demonstrate an increase in urinary prostaglan-

Table 3: Adenylate Cyclase Response* to Arginine Vasopressin (AVP) and Sodium Fluoride (NaF) in Rat Renal Outer Medullary Collecting Tubules and Ascending Limbs of Henle

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>10⁻⁴ AVP</th>
<th>NaF</th>
<th>X†</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Outer medullary</strong></td>
<td></td>
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<tr>
<td>Collecting tubule</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Control (n = 11)</td>
<td>183.4</td>
<td>1,872.2</td>
<td>9.3</td>
<td>3,875.5</td>
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<td>±4.35</td>
<td>±671.9</td>
<td>±3.1</td>
<td>±768.2</td>
<td>±5.0</td>
</tr>
<tr>
<td>Early escape (n = 5)</td>
<td>124.9</td>
<td>1,782.1</td>
<td>14.0</td>
<td>3,499.6</td>
</tr>
<tr>
<td>±11.5</td>
<td>±530.8</td>
<td>±3.7</td>
<td>±410.8</td>
<td>±6.1</td>
</tr>
<tr>
<td>Ascending limb</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n = 9)</td>
<td>52.9</td>
<td>1,028.4</td>
<td>21.7</td>
<td>3,163.0</td>
</tr>
<tr>
<td>±7.0</td>
<td>±326.0</td>
<td>±6.0</td>
<td>±601.4</td>
<td>±13.4</td>
</tr>
<tr>
<td>Early escape (n = 5)</td>
<td>74.2</td>
<td>1,059.1</td>
<td>13.9</td>
<td>3,299.1</td>
</tr>
<tr>
<td>±16.1</td>
<td>±370.2</td>
<td>±3.7</td>
<td>±798.4</td>
<td>±8.5</td>
</tr>
</tbody>
</table>

* Expressed as fmol/µg protein per µm 30 min.
renal perfusion pressure, and renal blood flow. This index (Gross et al., 1982), mean arterial pressure, and arterial blood flow were increased with subsequent increases in cardiac output. Moreover, positive water balance observed in the present study resulted in a 13-15% increase in measured plasma volume (Gross et al., 1982) with an increase in urinary prostaglandin E2 excretion. We cannot determine whether this increased urinary prostaglandin E2 excretion was due to increases in renal synthesis or washout of preformed prostaglandin E2. To determine the physiological significance of the increased urinary prostaglandin excretion, water metabolism was studied in indomethacin-treated animals. The dose of indomethacin used (1.75 mg/kg per day) prevented the effect of hypotonic fluid and desmopressin to increase urinary prostaglandin E2 excretion while maintaining urinary prostaglandin E2 excretion at levels not different from control animals. In indomethacin-treated animals, desmopressin resulted in higher net water retention and a lower steady state plasma sodium concentrations when indomethacin-treated animals were compared with animals not treated with indomethacin. In preliminary studies by Campbell et al. (1982), escape from AVP was associated with an increase in urinary prostaglandin excretion and indomethacin appeared to retard this escape. However, in the present studies, animals receiving desmopressin and indomethacin were able to more efficiently escape from desmopressin. Together, these observations suggest that while prostaglandin E2 contributes to escape from desmopressin, escape can occur even when urinary prostaglandin E2 is maintained constant.

Since prostaglandin-independent factors appeared important in escape from desmopressin, we undertook additional studies. In this regard, it is well known that numerous intrarenal factors, such as glomerular filtration rate, renal blood flow, osmolar excretion, and interstitial toxicity, modulate the hydro-osmotic effect of vasopressin (Abbrecht and Malvin, 1961; Anslow and Wesson, 1955; Atherton et al., 1971; Chan, 1971; de Wardener and DelGreco, 1955). We therefore evaluated osmolar excretion and escape from desmopressin could be demonstrated in the absence of any change in osmolar excretion. In addition, inner medullary toxicity was never significantly lower in animals undergoing escape from desmopressin when compared with control animals. Thus, in the present model, escape could not be attributed to either an increase in osmolar excretion or a decrease in inner medullary interstitial toxicity with a resultant decrease in the gradient for water to diffuse out of the collecting duct fluid.

Another factor that could be responsible for the diminished hydro-osmotic effect of desmopressin seen in the present studies is a high flow rate of collecting tubular fluid. Thus, the renal vasodilation and high glomerular filtration rates observed in the present study could increase delivery of tubular fluid to the collecting duct. In vivo rat studies indicate clearly that high urinary flow rates prevent osmotic equilibrium between collecting duct fluid and renal interstitium despite large doses of vasopressin (Lote and Snape, 1977). The findings of the present study are compatible with a flow rate-dependent mechanism in the attenuation of the hydro-osmotic effect of desmopressin.

We also undertook studies on renal tissues cyclic 3'5'-adenosine monophosphate (cAMP) system components. These studies were performed since it is generally accepted that the effect of vasopressin to promote water movement in collecting tubule is mediated by adenylate cyclase activation of cAMP (Dousa and Valtin, 1976). Moreover, high concentrations of vasopressin in vivo may result in diminished in vitro stimulation of rat renal medullary adenylate cyclase. These observations suggest that the renal tissue adenylate cyclase may be down-regulated after exposure to high doses of AVP (Rajerson et al., 1977). Finally, recent studies demon-

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**Table 4**

<table>
<thead>
<tr>
<th>Condition</th>
<th>cAMP phosphodiesterase (pmol/μg protein per min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Outer medulary collecting tubule</td>
</tr>
<tr>
<td>Control (n = 10)</td>
<td>165.7 ± 9.4</td>
</tr>
<tr>
<td>Early escape (n = 5)</td>
<td>146.5 ± 3.2</td>
</tr>
</tbody>
</table>
strate that the diminished hydromotic effect of vasopressin that follows prolonged exposure of the toad urinary bladder to vasopressin is due in part to decreased adenylate cyclase activation (Handler, 1981). In the present studies, chronic exposure to DDAVP without water retention did not result in escape. We also measured adenylate cyclase activity in membrane fragments obtained from inner and outer medulla. No differences in either basal or stimulated adenylate cyclase levels were observed when control and experimental animals were compared early and late after escape. We also undertook studies on adenylate cyclase activity in dissected tubules. These studies were performed since vasopressin-sensitive adenylate cyclase is present both in rat collecting tubule and thick ascending limb (Imbert-Teboul et al., 1978). In rat outer medulla, ascending limbs out-number collecting tubules by a factor of 5:1 (Knepper et al., 1977). In these dissected tubule studies, similar basal and vasopressin-stimulated adenylate cyclase values were observed in control and experimental animals during early escape in both outer medullary collecting tubules and thick ascending limbs. Although the present biochemical studies do not support a pathogenetic role for diminished adenylate response to vasopressin in the escape phenomenon, additional dose-response studies will be required to clarify this issue.

In summary, the present studies suggest that water retention rather than chronic exposure to desmopressin is essential for escape from the hydromotic effect of desmopressin. The early phase of escape is associated with increased urinary prostaglandin E₂ excretion, and, when prostaglandin E₂ is inhibited, the onset of escape is delayed and a greater degree of water retention occurs. Increases in plasma volume, cardiac index, mean arterial pressure, renal blood flow, and glomerular filtration rate occur during the later phase of escape. Neither a decrease in renal interstitial solute concentration nor an increase in osmolar excretion is necessary for escape from desmopressin. We conclude that PGE₂ plays a role in initiating escape early after desmopressin. During the later phases of escape, plasma volume expansion results in a prostaglandin E₂-independent increase in renal blood flow, glomerular filtration and tubular flow rates which may play a role in the maintenance of the escape phenomenon.

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