Temporal delays and individual variation in antidiuretic response to desmopressin

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Juul KV, Erichsen L, Robertson GL. Temporal delays and individual variation in antidiuretic response to desmopressin. Am J Physiol Renal Physiol 304: F268–F278, 2013. First published November 7, 2012; doi:10.1152/ajprenal.00502.2012.—This study aimed to estimate the relationship between pharmacokinetics and the antidiuretic effect of desmopressin. In the investigator-blind, randomized, parallel group study, 5 dose groups and 1 placebo group, each consisting of 12 healthy, overhydrated, nonsmoking male subjects 18–55 yr of age were infused intravenously over 2 h with placebo or 30, 60, 125, 250, and 500 ng desmopressin in 50 ml of normal saline. Plasma desmopressin and urine osmolality rose by variable amounts during the infusions of 60, 125, 250, and 500 ng desmopressin. Plotting mean urine osmolality against the concurrent mean plasma desmopressin yielded a temporal delay between pharmacokinetic (PK) and -dynamic (PD) responses in all dose groups. Using simulation from the indirect-response model, assuming a constant (4 ng/ml) desmopressin concentration, this delay between PK and PD was estimated at 4 h (10th–90th percentile: 1.8–8.1). Within each group, however, there were large individual variations (2- to 10-fold) in the magnitude and duration of the antidiuretic effect. The antidiuretic effect of intravenous desmopressin in water-loaded healthy adults varies considerably due largely to factors other than individual differences in pharmacokinetics. The antidiuretic effect is time as well as dose dependent and may be self-amplifying. The most likely explanation for these findings is that the time required for a given level of plasma desmopressin to exert its maximum antidiuretic effect varies markedly from person to person due to individual differences in the kinetics of one or more of the intracellular mechanisms that promote the reabsorption of solute-free water by principal cells in renal collecting tubules.

DESMPRESSIN IS A SYNTHETIC analog of the naturally occurring antidiuretic hormone arginine vasopressin (AVP) that is used to treat pituitary diabetes insipidus (DI) (27), nephrogenic diabetes insipidus (NDI) (28), and severe diabetes insipidus (29), all of which are important for the use of desmopressin in physiological and pathophysiological research as well as clinical diagnosis and treatment. The aim of this study is to explore these questions in healthy adults who are water loaded to suppress interference in by endogenous AVP.

MATERIALS AND METHODS

Subjects

Seventy-two healthy, nonsmoking male subjects (18–55 yr of age) were recruited. The data from 12 subjects in the placebo group and from all 11 subjects in the 30-ng-dose group were analyzed but are not included in this analysis because they did not produce PK parameters and had little or no effect on urine osmolality. Two additional subjects (1 of 12 in the 500-ng group and 1 of 11 in the 125-ng group) were excluded from analysis because they developed nausea and vomiting, a potent stimulus for endogenous AVP release (25). Another subject in the 125-ng group was also excluded because of baseline hyponatremia, an indicator of some intrinsic abnormality in basal AVP secretion. Thus this analysis is based on the analysis of data from 43 male subjects assigned randomly to the 4 highest dose groups (Table 1). Their ages, body weights, and body mass indexes (BMI) in each group were similar both on average and in the range of individual variation (Table 1). The demographics in the placebo and 30-ng-dose group were similar.

Study Designs and Procedures

This was an investigator-blind, placebo-controlled, randomized, parallel group study. The subjects were admitted at the study center at 12 h predosing. The following morning, the subjects were requested to drink a volume of water corresponding to 1.5% of their body weight over a 30-min period. This water-loaded model is generally recognized as suitable for investigating antidiuretic response of V2R agonists. The protocol stipulated that subjects who developed nausea or vomiting were excluded and replaced since nausea and vomiting increase endogenous AVP release (25). The overhydration process started 2 h before dosing. The subjects were asked to void every 15 min and instructed to drink equivalent volumes of water to maintain a constant level of slight overhydration. When the urinary output rate exceeded 10 ml/min, which usually occurred 90–120 min after the start of the water load, the subjects received either drug or placebo, administered as a constant-rate intravenous (iv) infusion of placebo or desmopressin over 2 h. Five groups received either 30, 60, 125, 250, or 500 ng of desmopressin. These doses and method of administration were chosen to simulate the pharmacokinetic patterns observed after oral administration, of 10, 20, 40, 80, and 160 μg of the oral lyophilisate, the preparation and dose range most commonly used clinically for diagnosis or treatment.
The subjects were monitored for 6–12 h after the start of the infusion depending on the duration of the effect on urine osmolality. Urine was collected every 15 min, and the volume was replaced with an equal volume of water by mouth. If the urinary output rate had not returned to baseline 12 h after the start of infusion (i.e., exceeded 10 ml/min), the subject would be instructed to restrict the fluid intake and reevaluated the following morning.

Blood pressure (systolic and diastolic) and pulse rate were measured with an automatic blood pressure/pulse-measuring device (Boso Medicus). Blood pressure (systolic and diastolic) and pulse were recorded in the morning of admission, and hourly during the postdosing overhydration process to validate whether there was a fall in arterial blood pressure, indicating endogenous AVP release.

Serum sodium was measured before the start of infusion (i.e., 0–30 min predosing), at dosing (0–30 min predosing) and every hour until overhydration was stopped, but for a maximum of 12 h after the start of infusion. For safety reasons, the subjects stayed at the clinic overnight. Serum sodium was measured the following morning, and the subjects were discharged, unless serum sodium (or other parameter/sign/symptom) showed a clinically relevant deviation.

The study was approved by the ethics committee, the Declaration of Helsinki was followed, and informed consent was obtained from all healthy volunteers.

**Study Restrictions**

The subjects were advised to avoid excessive physical activity for 7 days preceding the study and during the study. Products containing caffeine, alcohol, or grapefruit juice had to be avoided from 2 days before admission and during the study days. Three meals were served during the study day. The content of sodium was equal and standardized for all subjects.

Subjects should not have used prescribed medication or over-the-counter medication within 2 wk or five half-lives of the drug, whichever was longer, before the dosing day. No NSAIDs were allowed within 14 days before dosing. Concomitant medication was not allowed during the study days, except for paracetamol.

**Study Drug**

Desmopressin was obtained commercially in 1-ml single-use ampules containing 4 μg/ml. Sterile, physiological saline (0.9% NaCl), USP for injection was used for dilution to a volume of 50 ml.

**PK Blood Sampling and Laboratory Methods**

Blood sampling for PK determination was performed at predose (i.e., 0–30 min predosing), and 15, 30, 45 min, and 1, 1.5, 2, 3, 4, 6, 8, 10, and 12 h after dosing. A volume of 14 (2 × 7) ml was drawn at each time point.

**Estimating Osmolality of Missed Urine Collections**

The values of missed urine collections were imputed by a monotone linear function using the measured values of the collections obtained immediately before (N1) and after (N2) the missed sample collections. The calculations are based on the following assumptions. 1) Collection N1 was obtained by complete emptying of the bladder. 2) Subsequent collections were missed due to subject’s inability to void (not to loss of the collection). 3) The osmolality of each missed collection period changed by a constant amount (x) over the value of the preceding period. It increased when N2 was greater than N1 and
decreased when N2 was less than N1. Thus, when N2 was greater than N1, the first missed collection was assigned an osmolality of N1 + 1x, the second missed collection a value of N1 + 2x, and so on upward for each of the subsequent missed collections as well as for the 15-min period immediately before the time the next collection was obtained (N2). 4) Collection N2 was obtained by complete emptying of the bladder. It included all the urine produced during the periods in which the collections were not made due to an inability to void. The measured osmolality of N2 is therefore the average of all the urine produced during the period of missed collections plus the urine produced during the 15-min period immediately before the collection.

**Indirect-Response Model**

The observed delay between desmopressin concentration and urine osmolality results in time-dependent PK/PD relationship, e.g., the relation in the initial infusion phase is different from that in the terminal phase. To estimate a unique (time-independent) effect of a certain desmopressin concentration, the steady-state PK/PD relationship was estimated using population PK/PD modeling. An indirect response model was used assuming that the effect of desmopressin was to inhibit the “elimination” of urine osmolality according to a sigmoid I_{max} function. Model fit to mean observed urine osmolality is presented in Fig. 5. It has previously been shown that urine osmolality vs. desmopressin concentration over time is consistent with an indirect-response model (7, 23)

\[ \frac{dR}{dt} = k_{in} - k_{out} \cdot \left(1 - \frac{I(t)}{I_{max}}\right) \cdot R, \quad R(0) = k_{in}/k_{out} \]

\[ I(t) = 1 - \frac{C_{in}}{I_{C50} + C_{in}} \]

where \( k_{in} \) represents the zero-order constant for production of the response, \( k_{out} \) defines the first-order rate constant for elimination of response, \( R \) is the response variable representing the urine osmolality, \( C \) is the concentration of desmopressin in plasma, \( I_{max} \) represents the maximum inhibitory effect attributed to desmopressin, \( I_{C50} \) represents the concentration producing 50% of the maximum inhibition, and \( n \) is a sigmoidicity factor controlling the steepness of the concentration-response curve. The assumptions for applying the indirect-response model is that the measured change in response is produced by indirect mechanisms of desmopressin action in vivo as desmopressin mediates its antidiuretic response by activation of V2Rs, and subsequent increase in water permeability in the kidneys by incorporation of aquaporins in the tubuli cells. Maximal achievable urine osmolality, \( O_{max} \), was not estimable for all dose levels and was therefore assumed identical for all subjects. This was implemented using the restriction \( I_{max} = 1 - k_{in}/k_{out} \cdot O_{max} \).

PK/PD modeling was carried out using nonlinear mixed-effects modeling software NONMEM version 7.1. A two-compartment PK model with random subject effects on primary PK parameters (clearance, intercompartmental clearance, central and peripheral volume of distribution) was used to describe the population mean and individual time-concentration profiles of desmopressin plasma concentration. Subsequently, an indirect-response model with desmopressin inhibiting osmolality decrease according to a sigmoid \( E_{max} \) function and random subject effects on all primary PD parameters (\( K_{in}, K_{out}, E_{max}, \) and sigmoidicity \( n \)) was used to describe the time-concentration urine osmolality profile using the individual (post hoc) PK parameter estimates as fixed input to the PD model. An additive-error model was used for the PD, and a combined additive/multiplicative-error model was used for the PK model. For both PK and PD, a first-order conditional estimation algorithm was used (with interaction for the PK model).

**Statistical Analysis**

The mean and range of individual plasma desmopressin and urine osmolality values at each collection period were determined and presented for each dose group up to the time that measurements were made in all subjects. The area under the curve (AUC) for each variable was determined for each subject by adding the values from time 0 until the end of the measurements in that subject. Correlations between variables were evaluated by standard least square regression analysis.

Other statistical analyses were based on population PK/PD modeling (see details below) as well as descriptive noncompartmental analysis.

Demographics included age, weight, and BMI. Mean plasma desmopressin (pg/ml) during and after iv infusions of 60, 125, 250, and 500 ng was plotted, as well as temporal changes in urine osmolality (mosmol/kgH2O) during and after the desmopressin iv infusions at same dose levels. For all dose levels, a two-compartment PK model was used to estimate clearance, total volume of distribution, and terminal half-life. The PK parameters were subsequently fixed and used as input to the indirect response PD model for urine osmolality. The mean and range of serum sodium values were reported for prehydration, posthydration, at intervals during and after infusion.

**RESULTS**

**Plasma Desmopressin**

Plasma desmopressin rose to detectable levels in all subjects during the 2-h infusions of 60, 125, 250, and 500 ng of the peptide (Fig. 1). The extent and therefore the rate of rise were proportional to the dose (Table 1). The maximum level (\( C_{max} \)) occurred at or within 15 min of the end of the infusion in all subjects. Within each group, however, the individual \( C_{max} \) values varied over a two- to threefold range. These variations did not correlate with the age or body weight of the subjects. When the infusions ended at 2 h, plasma desmopressin began to fall in all subjects in every dose group (Fig. 1). The time from \( C_{max} \) to half-\( C_{max} \) (\( t_{1/2} \)) averaged between 80 and 120 min in all groups with no discernible dose effect. Within each group, the individual \( t_{1/2} \) varied over a twofold range, and these variations did not correlate with age, weight, or \( C_{max} \). The total washout time, here defined as the time from the start of the infusion to the time that plasma desmopressin fell below the limit of detection (0.8 pg/ml) differed with the dose, ranging from an average of 4 h for the 60-ng group to 11 h for the 500-ng group (Fig. 1, Table 1). It also varied two- to threefold within each group, and the individual variations did not correlate with the subjects’ age or weight. However, they did correlate weakly with the \( t_{1/2} \) (\( P < 0.05 \)) in the 60-, 125-, and 250-ng-dose groups. Similarly, the average AUC in each group correlated directly and proportionately with the dose, but individual values within each group differed by two- to threefold (Table 1). The individual differences in AUC did not correlate with individual differences in age, body weight, or BMI in any dose group except the 60-ng group, where the AUC correlated weakly and negatively with age (\( P < 0.05 \)).

**Urine Osmolality**

After water loading of subjects, basal urine osmolality averaged ~70 mosmol/kgH2O in all groups and was <150 mosmol/kgH2O in every subject (Fig. 1). During the infusion of desmopressin, it rose in each subject. The average in each group by the end of the infusion differed with the dose, ranging
from 350 mosmol/kgH$_2$O in the 60-ng group to 700 mosmol/kgH$_2$O in the 500-ng group (Table 1). Individual variation, however, was enormous within each group not only in the level achieved (Fig. 1, Table 1) but also in the time course profile of the rise (Fig. 2). In some subjects, particularly those receiving the two lowest doses, the rise was erratic, with intermittent peaks and valleys (Fig. 2). In some subjects in the 60-, 125-, and 250-ng-dose groups, urine osmolality did not even reach 200 mosmol/kgH$_2$O by the end of the 120-min infusion whereas in others it rose as high as 600–1,000 mosmol/kgH$_2$O at the same time point. These variations did not correlate with the subjects’ weight, age, baseline urine osmolality, and baseline serum sodium or plasma desmopressin at the end of the infusion.

After the infusions ended, urine osmolality continued to rise in most subjects of the 125-, 250-, and 500-ng-dose groups even though plasma desmopressin had begun to fall (Fig. 1). The magnitude of the postinfusion rise differed with the dose, averaging 31, 102, 139, and 191 mosmol/kgH$_2$O in the 60-, 125-, 250-, and 500-ng-dose groups, respectively (Table 1). Expressed as a percentage of urine osmolality at the end of the infusion, these increments averaged 9, 26, 22, and 27% in the 60-, 125-, 250-, and 500-ng-dose groups, respectively. However, individual variation within each group was relatively large, especially among the subjects receiving the two lower doses. These variations did not relate to any other variables except the levels of osmolality at the end of the infusion in the 500-ng-dose group, and that relationship was strongly negative ($r = -0.90534, P < 0.001$). Thus the higher the rise during the infusion the smaller the rise in the postinfusion period, indicating that the maximum antidiuretic effect or “ceiling” was reached in most of the subjects in the 500-ng group. The duration of the postinfusion rise, calculated as the average of time elapsed from the end of the infusion to the maximum urine osmolality in each subject, also appeared to increase with the dose, ranging from 21 min in the 60-ng-dose group to 145 min in the 500-ng-dose group (Table 1). Again, however, the individual variations within each dose group were

![Fig. 1. Changes in plasma desmopressin and urine osmolality during infusions of different amounts of desmopressin in healthy water-loaded adult men. Mean and range of individual values are shown respectively by heavy dark line between lighter lines above and below. A–D: plasma desmopressin (pg/ml) at 60, 125, 250, and 500 ng. E–H: urine osmolality (mosmol/kgH$_2$O) at 60, 125, 250, and 500 ng.](http://ajprenal.physiology.org/DownloadedFrom/10.1152/ajprenal.00502.2012)
relatively large (Table 1) and unrelated to the age, body weight, basal urine osmolality, or basal serum sodium of the subjects.

After peaking, urine osmolality declined to $200 \text{mosmol/kgH}_2\text{O}$ in all subjects in whom monitoring was continued for the full 12 h (Fig. 1). As would be expected, the time it took for urine osmolality to decrease from its peak to $200 \text{mosmol/kgH}_2\text{O}$ also increased with the dose, ranging from an average of 66 min in the 60-ng group to 290 min in the 500-ng group (Table 1). Thus the total duration of action from the start of the infusion was also dose dependent, ranging from an average of 186 min in the 60-ng group to 410 min in the 500-ng group. Like the other pharmacodynamic properties, individual variation within each dose group was very large and these differences did not relate to the subject’s age, body weight, or basal serum sodium but they correlated positively with individual differences in the maximum level of urine osmolality achieved in the 60-ng ($r = 0.71$), 125-ng ($r = 0.94$), and 250-ng ($r = 0.68$) groups. The average AUC for urine osmolality varied directly with the dose (Table 1) but tended to plateau at the higher doses, indicating approximation to the maximum antidiuresis possible in these water-loaded subjects. The AUC values within each group, however, varied enormously and did not correlate with individual differences in age, weight, or BMI except in the 250-ng group where they correlated weakly and negatively with weight ($r = -0.6416394, n = 11, P < 0.05$) and BMI ($r = -0.691653, n = 11, P < 0.05$).

Relationship of Urine Osmolality to Plasma Desmopressin

As would be expected from the time course data (Fig. 1), the relationship between the average urine osmolality and the concurrent plasma desmopressin differed over the course of the study (Fig. 3). During the infusion, when plasma desmopressin was rising, urine osmolality also rose but the relationship between the two variables differed markedly depending on the dose. As the dose and therefore the rate of rise of plasma desmopressin increased, the relationship to urine osmolality was shifted to the right, indicating a lesser effect at any given level of the agonist. During the second phase, after the infusion ceased, the relationship in the 125-, 250-, and 500-ng-dose groups reversed direction as mean urine osmolality continued to rise while plasma desmopressin decreased (Fig. 3). The slopes of the relationship in this phase were similar in the three groups, but the positions again differed depending on the dose. A distinct second phase was not observed in the 60-ng-dose group. In the third phase, mean urine osmolality began to fall steeply in conjunction with further decreases in plasma desmopressin until both variables returned to near basal levels. At this time, the relationships between the two variables were more similar in the four groups, but they again appeared to be shifted slightly to the right at the higher doses (Fig. 3).

This shift to the right was also evident in the average level of plasma desmopressin found at both the beginning and end of the period in which urine osmolarity fell from maximum to $200 \text{mosmol/l}$ (Table 1). At both time points, individual variations in plasma desmopressin were extremely large in all four groups, and they did not correlate with the concurrent level of urine osmolarity except in the 500-ng-dose group at the beginning of the fall ($r = 0.6729$). At the end of the fall, the individual variations in plasma desmopressin did correlate significantly with the time it took to reach this endpoint in three of the groups, but all of the relationships were negative ($r = -0.74, -0.76, \text{ and } -0.77$ in the 60-, 250-, and 500-ng-dose groups, respectively). In other words, the longer it took for the effect of desmopressin to cease, the farther plasma desmopressin had fallen. Also of particular note, although the average duration of action of desmopressin increased with the dose, individual variations were very large and they did not correlate with individual differences in the $C_{\text{max}}$, $t_{1/2}$, or wash-out time for plasma desmopressin in any of the four dose groups.

Fig. 2. Individual urine osmolality (mosmol/kgH$_2$O) profiles in 4 subjects infused with different doses of desmopressin by dose.

Fig. 3. Antihysteresis plot of median desmopressin concentration (pg/ml) vs. urine osmolality (mosmol/kgH$_2$O) by dose.

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groups. The AUC for urine osmolality was closely related to the AUC for plasma desmopressin ($r = 0.83, P < 0.001$) when the individual values from all four dose groups were combined (Fig. 4). However, there was also considerable scatter around the line, and a breakdown by dose shows no significant correlation between the two values except in the 500-ng group ($r = 0.7511, P < 0.05$).

**Serum Sodium**

Two hours after the water load when urinary dilution had occurred, serum sodium averaged $\sim 137$ mmol/l in all four groups (Table 1). The individual values ranged from $\sim 133$ to 140 mmol/l except for one subject in the 125-ng-dose group, who had a basal level of 127 mmol/l (Table 1). This subject was excluded from the analysis because hyponatremia before desmopressin infusion suggested some intrinsic abnormality in antidiuretic function. After the infusions began and urinary concentration occurred, the average serum sodium declined gradually in all groups and the individual values remained at or below the preinfusion level in every subject, confirming continued suppression of endogenous vasopressin. There was no correlation between mean serum sodium during the study and the AUC for plasma desmopressin or urine osmolality in any dose group.

**Blood Pressure and Pulse Rate**

No abnormal findings of clinical relevance were observed for vital signs (systolic blood pressure, diastolic blood pressure, and pulse rate) during the study, and no vital signs findings were classified by investigator as adverse events.

**PK/PD Modeling of Desmopressin and its Effect on Urine Osmolality**

**PK modeling results.** The PK of desmopressin after intravenous infusion of 60, 125, 250, and 500 ng was described for all dose levels by a two-compartment model with estimated median (10th-90th percentile) clearance: 8.9 (5.9–11.2) l/h, total volume of distribution: 12.9 (6.2–22.7) liters, and terminal half-life: 3.8 (2.8–5.7) h. Observed $C_{\text{max}}$ (maximal concentration) and $AUC_{t}$ (area under the concentration time curve from 0 to last observation) were approximately dose proportional with (log-log) regression slope (log-dose as covariate) equal to 0.85 (SE = 0.04) and 1.25 (SE = 0.09) for $C_{\text{max}}$ and $AUC_{t}$, respectively.

**Model fit.** The model fit to mean PK curves and to mean observed urine osmolality is shown in Fig. 5, both indicating a reasonable agreement between the model and observed data, which also is the case for individual curves.

**Urine osmolality modeling results.** The estimated (steady state) relationship between desmopressin concentration and urine osmolality is shown in Fig. 6, with maximal inhibition and IC$_{50}$ estimated at 0.93 (0.90–0.96) and 1.14 (0.46–2.28), respectively. Maximal achievable urine osmolality ($O_{\text{max}}$) was estimated at 947, 423 (105–970), and 117 (45–401) mosmol/kg$_{\text{H2O}}$ for steady-state plasma desmopressin levels of 4, 2, and 1 pg/ml, respectively (Table 2).

**Delay between PK and PD.** Delay between PK and PD is illustrated in Fig. 7 and Table 2 using simulation from the indirect-response model assuming a constant (4, 2, and 1 ng/ml) desmopressin concentration. The median (10th-90th percentile) delay is estimated as time to 95% of maximum
urine osmolality yielding 4.0 (1.8–8.1), 2.0 (0.5–6.7), and 0.5 (0.0–2.3) h for steady-state plasma desmopressin levels of 4, 2, and 1 pg/ml, respectively (Table 2).

**DISCUSSION**

This study shows that the antidiuretic effect of desmopressin in healthy, water-loaded adult men is determined not only by dose and PK but also by time and some other factor(s) that differs from person to person. The time dependency is indicated by the extended, postinfusion rise in urine osmolality in subjects infused with 125, 250, and 500 ng of desmopressin (Fig. 1). Its occurrence in all three dose groups indicates that it is not due to continued stimulation by supramaximal levels of desmopressin. Therefore, it must be the delayed effect of some antidiuresis-enhancing mechanisms set in motion but not completed during the infusion. The magnitude and duration of this delayed effect seem to be dose dependent because it was observed in only a few subjects infused with 60 ng of desmopressin and increased with the dose in the other groups.

The time dependency of the full antidiuretic effect is also evident during the infusion when the relationships between mean urine osmolality and the concurrent plasma desmopressin in each of the groups are compared (Fig. 3). The progressive shift to the right in the relationship as the rate of infusion increased indicates that the magnitude of the effect of a given level plasma desmopressin depends not only on the level of plasma desmopressin produced but also on how fast that level is reached: the slower the rise in plasma desmopressin, the greater the effect at any given level. The length of the delay in achieving the full effect cannot be determined directly from the observed results because plasma desmopressin rose and fell continuously during the study. Using simulation from the indirect-response model (Fig. 7), the equilibration delay between steady-state plasma desmopressin concentration of 4 pg/ml and a maximum urinary osmolality of ~900 mosmol/kgH2O is estimated to average 4.0 h but with considerable individual variation (1.8–8.1 h). This result agrees well with a previous study in which a moderately high dose of desmopres-
sin (396 ng) was given by bolus injection to water-loaded, healthy subjects (7) and is close to the average time elapsed from the start of the infusion to peak effect (265 min) in the group infused with 500 ng of desmopressin (Fig. 1, Table 1). Our data and the indirect simulation model also indicate that not only the magnitude of the maximum antidiuretic effect but also the time delay in reaching it decrease with the dose (Tables 1 and 2).

The reason(s) for the delay in producing the maximum rise in urine osmolality is not clear. It could be due in part to the time required for newly formed urine to traverse the renal pelvis and ureters into the bladder. However, this is unlikely to be a major contributor because the volume of these spaces is relatively small and more potential than actual (9). Another possibility is that the concentration of desmopressin at renal V2R differs from that in plasma due to the existence of some barrier that retards its diffusion into and out of its site of action. However, such a barrier has not been described, and its existence seems very unlikely given how quickly the response begins. The more likely explanation for the significant temporal delay in producing the full response is the time required to fully activate and then deactivate the various cellular mechanisms that mediate, enhance, or diminish the antidiuretic effect of V2R stimulation.

The antidiuretic effects of V2 agonists are mediated by promoting the reabsorption of water from modified glomerular filtrate when it reaches the collecting ducts of the kidney (20, 22). This reabsorption is achieved via binding of the agonist to V2R on the serosal surface of principal cells followed by activation of adenylate cyclase, increasing the production of cAMP. The latter, in turn, stimulates phosphokinase A which phosphorylates aquaporin-2 (AQP2) water channels, increasing their association into tetramers and insertion through the luminal surface of the principal cells. This, in turn, permits water to back diffuse through the cells down the osmotic gradient created by the hypertonic milieu of the renal medulla (22). Studies in animals indicate that the increase in AQP2 in the membranes occurs rapidly, anywhere from 5 to 15 min after administration of AVP or desmopressin (18): this is attributed to acute trafficking of a preformed pool of AQP2 to the membrane, or phosphorylation of a pool of AQP2 already present in the basolateral membrane. If these kinetics are representative of the rate of trafficking of AQP2 in healthy humans, they do not explain the relatively long delay in achieving the full antidiuretic effect in the present study.

However, stimulation of V2R also sets in train a number of other cellular events in cortical and medullary collecting tubules that probably take longer and serve to enhance the magnitude and/or duration of the antidiuretic effect (2, 3, 6, 12, 24, 28, 29). They include increased synthesis as well as decreased endocytosis and degradation of AQP2 as well as increased tubular reabsorption of urea and sodium (2, 11),

Table 2. Time delay to maximum antidiuretic effect obtained by simulation with indirect-response model for different steady-state levels of plasma desmopressin

<table>
<thead>
<tr>
<th>Variable</th>
<th>Modeled Steady-State Levels of Plasma Desmopressin</th>
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<tr>
<td></td>
<td>1 pg/ml</td>
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<tr>
<td></td>
<td>Median 10th–90th Percentile</td>
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<tr>
<td>Time delay between PK and PD using simulation from the indirect-response model, h</td>
<td>0.5 0.0–2.3</td>
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<tr>
<td>Maximum antidiuretic effect, mosmol/kgH2O</td>
<td>117 45–401</td>
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Results are shown as median and 10th–90th percentile (80% prediction interval).
which could further increase water reabsorption by reducing the urinary solute load and increasing the hypertonicity in the renal medulla. The hypertonicity, in turn, may also feed back positively to further enhance the expression of V2R and AQP2 (14), thereby increasing even more the effectiveness of the system. In addition to phosphokinase A, V2 agonists also increase the phosphorylation of many other proteins whose role if any in antidiuresis is not yet known (13). The kinetics of these effects and their contribution, if any, to the observed delay in full activation and deactivation of the antidiuretic effect of desmopressin are unknown. However, it is conceivable if not likely that each of them is dose dependent and/or come to maximum fruition at different rates in different people. This could account not only for the delay in achieving the full effect but also the surprisingly large individual variation in the rate and/or magnitude of the response and the fluctuations in urine osmolality observed over time in some subjects given small to intermediate doses (125 ng) of desmopressin (Fig. 2).

Cellular mechanisms for deactivating or constraining the antidiuretic response to V2 agonists may also play a role in determining the duration and magnitude of the extended rise in urine osmolality. The contribution of these mechanisms to our findings cannot be determined because plasma desmopressin was continuously changing, and a method for modeling the delay in reaching steady-state effects when desmopressin falls to zero is not available. However, the V2R is subject to homologous desensitization, probably by internalization, and this desensitization may begin within 1–2 h of exposure (5). If this desensitization is dose dependent, it might contribute to the dose-related shift to the right in the relationship of urine osmolality to plasma desmopressin during the third phase of the study (Fig. 3, Table 1). The antidiuretic effect of V2 agonists may also be constrained by one or more post-receptor intracellular mechanisms. In any case, the kinetics of these desensitization or modulating processes, including the removal of AQP2 from the membrane, may be as important as PK in determining the duration of action of desmopressin and other V2R agonists.

Another issue raised by this study is whether the use of water loading to suppress endogenous vasopressin caused or increased the delay in producing the full response to desmopressin. It is known that a chronic deficiency of vasopressin due to pituitary DI or primary polydipsia diminishes the acute antidiuretic response to vasopressin (10, 32). However, a previous study showed that acute water loading to suppress endogenous vasopressin did not alter the PK or PD of a bolus injection of desmopressin (7). We also found that individual variations observed in this study did not correlate with differences in serum sodium before or after the water load. We conclude, therefore, that our findings were not appreciably influenced by the water load. Nevertheless, it would be of interest to repeat these studies in patients with severe pituitary DI, where a reduction in serum sodium by water loading is not needed to eliminate the influence of endogenous vasopressin.

This study also revealed very large individual differences in the antidiuretic response to desmopressin. These individual variations involved all facets of the response and were as evident in the results of the simulated indirect model as in the raw data itself. They could not be explained by differences in collection, corrections for missing values, fluctuations in the response curves, or the time course of the response because they were as large or even larger when the effects were expressed as the AUC for urine osmolality (Table 1). The differences seemed to be greatest among subjects receiving the intermediate or lower doses (Figs. 1 and 2, Table 1), suggesting that they may be reduced or eliminated by higher doses of desmopressin. Indeed, in the subjects given 500 ng, the individual differences in AUC for urine osmolality correlated significantly and positively with the AUC for plasma desmopressin, suggesting that exposure was the primary if not the only determinant of the variable effect in that group. However, this correlation was not observed in the other three groups, indicating that some factor(s) other than exposure to desmopressin is of greater consequence when V2R stimulation is submaximal. The variations are unlikely to be due to release of endogenous vasopressin since serum sodium remained low throughout the study, blood pressure and pulse remained stable, and other known nonosmotic stimuli for vasopressin release, such as nausea, were not observed. They did not relate to age, body weight, BMI, or the average level of plasma sodium in the subjects. Large individual differences
also were observed in another similarly designed study in patients with pituitary DI (15), indicating again that water loading and/or release of vasopressin was not responsible. Therefore, we postulate that the individual differences in the antidiuretic response to low or intermediate doses of desmopressin are due largely to genetically determined differences in the kinetics of one or more of the many cellular mechanisms that mediate antidiuresis. The proposed mechanism is analogous to the large genetically determined differences in the vasopressin response to osmotic stimulation in healthy adults (23). This theory could be tested by determining whether the individual differences in the effect of desmopressin are reproducible and, if they are, whether they correlate significantly better in monozygotic than in dizygotic twins.

Since our study only included men, the suggested twin study should also include women to investigate whether previously reported gender differences in the antidiuretic response to desmopressin (16) also in gender difference in the temporal delay and the magnitude of individual variation in the antidiuretic response to desmopressin. The concept of a possible gender difference has recently been supported by the finding that female rats express significantly more V2R mRNA and protein in kidneys than males and that this results physiologically in a greater sensitivity to V2R agonist administration (17). The AVPR2 gene encoding V2R is located on the X chromosome, and it has been shown that the AVPR2 gene has a high probability of escaping X inactivation and hence may be responsible for gender differences in renal V2R expression (4, 8).

Although relatively small, the individual differences in PK in the present study were also surprising and difficult to explain because desmopressin was administered intravenously. These differences included not only the maximum concentration at the end of the infusion but the AUC. Neither variable correlated with individual differences in age or body weight and, therefore, seem unlikely to be due to differences in the volume of distribution. Other possibilities include differences in desmopressin metabolism and elimination, binding to plasma proteins, and even absorption of desmopressin onto the plastic syringes utilized in the iv administration. A recent analysis of two desmopressin trials in healthy volunteers (16) demonstrated no significant age- or gender-specific differences in the PK properties of desmopressin. However, in addition to dose proportionality, they also showed an inverse proportionality to weight that was significant for C_max and borderline significant for AUC. The reason for the discrepancy with the present study is unknown.

The findings of this study have several implications for clinical and basic investigations of antidiuretic function. One applies to the traditional use of desmopressin or vasopressin challenge tests for the differential diagnosis of DI. The previously noted difficulty in finding a level of response that reliably differentiates primary polydipsia from partial pituitary or partial nephrogenic DI (26) is now explicable by the large individual variation in the timing and magnitude of the antidiuretic response to desmopressin even when it is given by injection to healthy subjects. Fortunately, these problems in differential diagnosis can now be solved by newer more direct methods that use vasopressin assays and brain MRI or closely monitored 2-day trials of desmopressin to differentiate between the various types of DI (1). Another implication of the present study is that the PK of desmopressin do not always provide an accurate guide to the magnitude or duration of its antidiuretic effects. This may be particularly relevant to efforts to elucidate the cause of the hyponatremia that develops in a few children or elderly patients treated with desmopressin for control of enuresis or nocturia (30). In this regard, it will be of value to determine whether the large individual variations in the effect of desmopressin on urine concentration are due to differences in age, genetic, or environmental factors. The challenges for basic research will be to better define the kinetics of the various biochemical reactions that mediate, modulate, or turn off antidiuretic responses to desmopressin and to identify the genes that code for the critical components of the rate-limiting steps. In this regard, it will be important to bear in mind the influence of genetic differences, within as well as between species.

Conclusions

In summary, this study describes some important and little recognized characteristics of the antidiuretic action of desmopressin (and probably AVP itself). These characteristics might be summarized as a considerable lag or delay in exerting its full effect and large individual differences in response that cannot be explained fully by differences in PK. It may also have autocatalytic or self-enhancing effects that manifest as an apparent changing of renal “sensitivity” to the hormone. The delay is probably due to the different times required to fully activate all the various biochemical mechanisms that must contribute to achieve the maximum antidiuretic effect. The cause(s) of the relatively large individual differences in the magnitude or timing of the response is more difficult to identify but may involve preexisting environmental or genetic differences in basal plasma vasopressin or any of the cascade of cellular mechanisms that mediate its effects.

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DISCLOSURES

K. V. Juul and L. Erichsen are employees of Ferring.

AUTHOR CONTRIBUTIONS


REFERENCES


