Influence of plasma amino acid level on vasopressin secretion

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SUMMARY

Objectives: Vasopressin (VP) is known to be elevated in patients with diabetes mellitus (DM). While the influence of acute hyperglycemia has been ruled out, the mechanism or the osmotically active compound responsible for the increase in VP secretion is still not elucidated. Because the plasma level of several amino acids (AAs) is increased in DM, we evaluated whether AAs could represent an effective osmotic stimulus for VP secretion.

Research Design and Methods: In a cross-over study, eight healthy volunteers randomly received an infusion of isotonic saline (control) or mixed AA solution, i.v., at a low or a high rate (2 or 4.5 mg/min/kg BW, respectively). Plasma VP (PVP) was measured for two hours before and three hours during AA or control infusion.

Results: AA infusion induced a dose-dependent elevation in plasma AA concentration but did not alter PVP. However, effective plasma osmolality (Pposm) (osmolality minus urea concentration) remained unchanged because a concommitant fall in plasma sodium concentration (PNa), likely due to sodium-linked uptake of AA in peripheral cells, compensated for the rise in plasma AA.

Conclusion: The stability of effective Pposm may explain the lack of change observed in PVP. Because sodium is a very efficient stimulus for VP secretion, it may be assumed that the fall in PNa occurring during AA infusion should have reduced VP secretion and thus PVP. In this setting, the stability of PVP suggests that AAs induced an increase in VP secretion which counterbalanced the fall attributable to the decrease in PNa. In conclusion, in acute experiments, AAs seem to represent an effective stimulus for VP secretion.

Key-words: Diabetes Mellitus - Diuresis - Osmolar Clearance - Plasma Osmolality - Plasma Sodium Concentration.


RESUMÉ

Influence du taux d’acides aminés plasmatiques sur la sécrétion d’hormone antidiurétique

Objectifs : L’hormone antidiurétique ou vasopressine (VP) est élevée dans le diabète sucré (DM). Cette élévation se semble pas due à l’hyperglycémie et sa cause ou le composé osmotiquement actif impliqué ne sont pas encore identifiés. Le taux plasmatique de certains acides aminés (AA) est augmenté dans le DM et nous avons donc voulu déterminer si les AAs pouvaient être un stimulus efficace pour la sécrétion de VP.

Méthodes : Dans une étude en "cross-over", huit volontaires ont reçu, dans un ordre aléatoire, une perfusion i.v. de sérum salé isotonique (contrôle) ou d’un mélange d’acides aminés à un taux modéré ou élevé (respectivement 2 ou 4.5 mg/min/kg poids corporel). Le taux plasmatique de VP (PVP) a été mesuré pendant les deux heures précédant la perfusion d’AAs ou de solution contrôle et pendant les trois heures suivant cette perfusion.

Résultats : La perfusion d’AAs a produit une élévation dose-dépendante du taux plasmatique d’AAs mais n’a pas modifié PVP. Cependant, l’osmolalité plasmatique (Pposm) efficace (osmolalité moins l’urée) n’a pas non plus varié en raison d’une baisse simultanée de la natrémie (PNa), probablement due à une entrée sodium-dépendante d’AAs dans les cellules qui a compensé l’augmentation d’osmolalité due aux AAs.

Conclusion : La stabilité de la Pposm efficace peut expliquer l’absence de changement de PVP. Le sodium est un stimulus très puissant de la sécrétion de VP. La baisse de PNa qui s’est produite pendant la perfusion d’AAs aurait donc dû faire baisser PVP. La stabilité de PVP suggère que les AAs ont induit une augmentation de sécrétion de VP qui a compensé la baisse qu’a dû engendrer la chute de PNa. En conclusion, au cours d’expériences aiguës, les AAs semblent constituer un stimulus de la sécrétion de VP presque aussi efficace que le sodium. Des travaux complémentaires sont nécessaires pour déterminer l’influence des AAs dans la situation chronique caractéristique du diabète.

Mots-clés : Diabète sucré - Diurèse - Clairance osmolaire - Osmolalité plasmatique - Natrémie.

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More than twenty years ago, Zerbe et al. reported that patients with insulin-dependent diabetes mellitus (DM) and ketoacidosis had markedly elevated levels of vasopressin (VP) [1]. Subsequent studies in humans with non-ketotic uncontrolled DM confirmed this finding [1-6]. In human as in rat, correction of both ketoacidosis and hyperglycemia is accompanied by a significant reduction in plasma VP (P<sub>VP</sub>) [1-3, 5, 7].

As recently reevaluated in rats [8, 9] and humans [10, 11], the high P<sub>VP</sub> observed in DM appears to be an appropriate adaptation that limits the amount of water lost in the urine owing to glucose-induced osmotic diuresis. Although producing a large volume of urine with (moderately) diminished urine osmolality, the diabetic kidney exerts a considerable concentrating activity devoted to the concentration of glucose in the urine, as reflected by enhanced solute-free water reabsorption [8, 9, 11]. In the long term, this increased concentrating activity might be deleterious. Indeed, in humans and rodents, acute infusion of dDAVP induces a significant increase in urinary albumin excretion [12]. In rats, chronic infusion of dDAVP, a selective agonist of V2 vasopressin receptors has been shown to increase proteinuria and to accelerate the progression of renal failure induced by 5/6 nephrectomy [9, 13-15]. Finally, in diabetic rats, chronic blockade with a V2 receptor antagonist completely blunted the rise in albuminuria [16]. In the whole, these results suggest that vasopressin could play a role in the early stage of diabetic nephropathy [10].

What could be the factor responsible for increased VP secretion in DM? Hyperglycemia per se was first suspected. However, this hypothesis was ruled out when intravenous infusion of hypertonic dextrose was shown not to increase P<sub>VP</sub>. A resetting of infusion of hypertonic dextrose was shown not to increase P<sub>VP</sub> [17, 18]. However, this hypothesis was ruled out when intravenous infusion of hypertonic dextrose was shown not to increase P<sub>VP</sub> [17, 18].

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Besides glucose, amino acids (AAs) could represent an efficient osmotic signal for VP secretion in DM. Indeed, AA metabolism is perturbed in DM, and the plasma level of several AAs is modified in patients with diabetic ketoacidosis. Although all reports do not agree on the changes in individual AA, a distinct increase in whole aminoacidemia is usually present with a marked elevation in branched chain AAs and in a few additional AAs (among which lysine), while others, including gluconeogenic AAs, are reduced [22-27]. Moreover, plasma level of branched chain AAs exhibits a positive correlation with blood glucose [24, 25, 27]. Such dissociation may partly result from the role exerted by insulin on AA transport through cell membranes [28-31]. We therefore hypothesized that a chronic elevation in the plasma level of AAs could play a role in the high VP secretion of diabetes. This assumption also relies on the following observations. P<sub>VP</sub> rises significantly after a single protein meal in healthy subjects and in uremic patients [32, 33]. P<sub>VP</sub> is also elevated in healthy subjects chronically submitted to a high protein intake [34]. The rise in P<sub>VP</sub> occurring after a protein meal was positively correlated with the rise in plasma osmolality but not with the changes in plasma sodium or urea. Because plasma AA concentration is known to increase by 2-3 mmol/L and the rise in plasma osmolality could not be accounted for by sodium or urea, it was assumed that AAs were the most likely solutes that could trigger post-meal VP secretion [32].

In order to test whether plasma AA concentration influences VP secretion and may account for the elevation of VP in DM, we carried out a cross-over study in healthy volunteers who randomly received a 3-hour infusion of isotonic saline (control) or of saline plus a mixture of amino acids at either a low or a high dose so as to induce widely different plasma levels of AAs. Plasma VP, plasma osmolality and renal function were evaluated hourly before and during these infusions.

### Experimental subjects

The study was conducted in eight healthy male volunteers aged 22-27 yr (24.3 ± 0.7, mean ± SEM), weighing 58-81 kg (71 ± 3) and measuring 170-189 cm (180 ± 2). The protocol was approved by the Hôpital Necker-Enfants Malades Ethics Committee, and each subject gave written informed consent to participate in the study. Subjects were on a free diet and were not taking any medication in the last two weeks preceding the study. Urine was collected for the last 24 hours before each test for a posteriori control of daily fluid and solute excretion.

### Materials and methods

#### Study protocol

The study was conducted at the “Centre d’Investigation Clinique” at Hôpital Necker-Enfants Malades (Paris). All subjects underwent the three tests at 2-3 weeks intervals in a randomized order. They were infused with either isotonic saline plus a low dose of AA (Low-AA), isotonic saline plus a high dose of AA (High-AA) (see further), or isotonic saline alone (control). After completing all tests, data collected from one of the subjects was excluded a posteriori from analysis because of permanently high values of plasma VP (5-7 times above normal) and the discovery of a tumor of the central nervous system.
After an overnight fast, tests started at 8:00 am. Indwell-
ing canulas were placed in the right and left brachial veins (for infusions and blood sampling, respectively) and an i.v. infusion of isotonic saline was started at a rate of 1 ml/min and maintained for the entire test. A priming dose of inulin (Inutest, Laevosan, Linz, Austria) was given i.v., followed by a sustaining infusion (inulin being added at the appropriate concentration to the isotonic saline infusion) in order to evaluate glomerular filtration rate (GFR). Subjects were not allowed to drink or eat throughout the test. They were in the supine position and stood up only for voiding every hour. After an equilibration period of 60 min and an initial voiding (urine not collected), five consecutive clearance periods were performed consisting in two one-hour basal periods (saline infusion) and three one-hour experimental periods (infusion of AA + saline or saline alone) (Fig 1).

A blood sample (~ 25 ml) was drawn from the brachial vein before the start of inulin administration (blank), and then every hour, starting just at the beginning of the first urine collection and ending after completion of the fifth urine collection. For biochemical, inulin and insulin measurements, blood was collected in heparinized tubes, and for glucagon and VP measurements on aprotinin and EDTA, respectively. Before each blood sampling, thirst intensity was evaluated on a visual scale [20]. Immediately after blood collection, plasma was separated by centrifugation. Osmolality was measured immediately in plasma and urine. Plasma and aliquots of urine samples were frozen at -20°C for subsequent analysis.

Special care was given to avoid any perturbation in renal function. Therefore no water load was given before the experimental protocol and no oral fluid supply was allowed during the tests so as not to alter the vasopressin-fluid balance. The rate of isotonic saline infused i.v. was modest (1 ml/min) and remained unchanged throughout the three tests to avoid volume expansion and unequal fluid supply. This rate of isotonic saline infusion was shown to induce no change in VP secretion and not to interfere with the VP response to various osmotic stimuli ([20] and G. Robertson, personal communication). Renal blood flow, which is often approximated by the clearance of paraminohippurate, was not measured in this study because this marker is known to influence proximal tubular reabsorption [35, 36] and could interfere with the effects of AAs on renal function.

**Amino acid mixture**

A commercial mixture of AAs, (Azonutril 25®, Pharmacia, France) was infused in the experimental periods (i.e., for the last three hours of the tests). It consisted of branched-chain AAs (21%), arginine (17%), gluconeogenic AAs (38%) and other AAs (24%). It was infused, undiluted, at a rate providing per kg body weight either 2 mg/min (Low-AA test) or 4.5 mg/min (High-AA test), or no infusion was given (control). Just before each blood collection, subjects were asked to grade their thirst on a visual scale.

**Biochemical measurements**

Plasma and urinary VP concentrations were measured by radioimmunoassay as previously described [37, 38]. All plasma samples were assayed at the same time, within a single RIA. This radioimmunoassay can routinely detect concentrations of VP as low as 0.5 pg/ml [37]. Plasma from
patients with severe central diabetes insipidus were included and found to have undetectable VP level. The urinary samples were not extracted, were diluted, and were also measured within a single RIA.

The concentration of individual AAs in plasma was measured by ion exchange chromatography (Beckman System Analyzer 6300) and subsequent coloration by ninhydrine [39]. Plasma insulin and glucagon concentrations were measured by radioimmunoassays (Inskit 5, Sorin Diagnostics, and Glucagon-RIA, Biodata Diagnostics). Insulin concentration was measured by a colorimetric method adapted on Autoanalyzer I Technicon (Technicon Instrument Corp.) [40], and plasma and urine osmolality (P_{osm} and U_{osm}, respectively) with a freezing point osmometer (Microosmometer Roebling). The concentrations of the main electrolytes, glucose and urea in plasma and urine were determined on a multiparametric analyzer (Hitachi 917).

Calculations and statistical analysis

Urine flow rate and the excretion of total osmoles, main urinary solutes, and VP, as well as inulin clearance and solute-free water reabsorption (T\(\text{H}_2\text{O}\)) were calculated according to usual formulas. The “basal” period was taken as the mean of the two basal hours, and the “experimental” period as the mean of the last two hours of the AA or saline infusion (the first hour being considered as a transition period). The statistical analysis was performed with Statview 5, using one-way ANOVA with repeated measures followed by the Fisher post-hoc test.

Results

Changes induced in plasma AA concentration (P_{AA}) in response to AA infusion are depicted in figure 2 (top). A dose-dependent rise in P_{AA} was observed during the first hour and P_{AA} almost plateaued during the last two hours at about 1.8 mmol/L above baseline for the Low-AA test and 4.4 mmol/L for the High-AA test. Reaching a plateau despite continuous infusion demonstrates that progressive storage and/or metabolism of infused AA was equivalent to the rate of AA infusion. The increase in plasma concentration of arginine, lysine, leucine, and valine accounted for the largest fraction of the rise in P_{AA}. Their concentration rose 7.3-, 4.3-, 4.2-, and 3.9-fold, respectively. These 4 AA together represented 22.3% of total plasma AAs in the basal period and 39.6% at the end of the High-AA test. Glutamine, the most abundant AA in plasma (about 600 \(\mu\text{mol/L}\)), did not increase during AA infusion.

The changes observed in response to saline or AA infusions are displayed in Table 1. ANOVA revealed no significant differences between the basal periods of the three tests for any parameter. Accordingly, for the sake of simplification, basal values shown in Table 1 are pooled for the three tests. But each experimental period was compared to its own basal period in the statistical analysis. Most changes observed in experimental periods of the High-AA infusion rate reached statistical significance. During the Low-AA test, intermediate levels between the control test and the High-AA test were reached (Tab I).

As expected, AA infusion increased GFR (by 8% with Low-AA and 27% with High-AA) (Tab I). Both urinary flow rate and osmolality tended to increase after AA infusion, resulting in a significant rise in osmolar excretion (+59% with High-AA). T\(\text{H}_2\text{O}\) increased significantly after AA infusion with a similar time course as the change in osmolar excretion (Tab I and Fig 3). Plasma glucagon rose dose-dependently during AA infusion (+50% with Low-AA and +100% with High-AA, p < 0.001 for both). Insulin rose only modestly with the Low-AA infusion (+30%, NS) but more intensely with High-AA (+112%, p < 0.001) (Tab I). No change was observed in these two hormones during the control test. The time course of the rise in the two pancreatic hormones during AA infusion was strikingly different. In both tests, glucagon rose steadily during the three hours. In contrast, insulin, after rising during the first hour, remained stable for the last two hours.

Plasma glucose and plasma urea concentrations rose dose-dependently during AA infusion (in the High-AA test, +0.3 and +1.6 mmol/L, respectively) (Tab I), indicating that part of the amino acids were used for gluconeogenesis and ureagenesis (two closely associated metabolic pathways in the liver). The newly-formed urea was excreted by the kidney, resulting in almost a doubling of urea excretion during the High-AA test. The rise in urea excretion accounted for half the total increase in osmolar excretion during both the Low- and the High-AA infusions (Tab I). Sodium excretion also increased significantly (by more than one third during High-AA).

In spite of the marked elevation in P_{AA}, no change was observed in P_{VP} during AA infusion at either dose (Fig 2) while a dose-dependent rise in urinary VP excretion was observed (Fig 3). The changes in urinary VP excretion were not correlated with the simultaneous changes in urine flow rate (not shown) but exhibited a significant correlation with the changes in osmolar excretion (p < 0.01). Of note, the thirst sensation, which increased with time in the control test, increased more intensely, and dose-dependently, during AA infusion (Tab I).

The increase in P_{osm} did not parallel the rise in P_{AA} (+1.8 mosmol/kg \(\text{H}_2\text{O}\) for P_{osm} as compared to +4.4 mmol/L for P_{AA} during the High-AA test). This is explained by the simultaneous, dose-dependent fall in plasma sodium concentration (P_{Na}) (-1.6 mmol/L during the High-AA test, p < 0.01) (Tab I). Plasma chloride concentration remained unchanged. The fall in P_{Na} was significantly and negatively correlated with the rise in P_{AA} occurring simultaneously in the three tests (Fig 4). Together with the fall in associated anion(s), this fall in P_{osm} should have decreased P_{osm} by
that in Posm.

1.6 x 2 = 3.2 mosmol/kg H2O, a value which approximately corresponds to the “gap” seen between the rise in PAA and that in Pvsw.

Discussion

In this study, we attempted to evaluate whether changes in plasma amino acid concentration influence vasopressin secretion in healthy subjects. Amino acid infusion at two different rates induced the well-known changes in GFR [41-43] and in glucagon and insulin secretion [41, 44]. They also resulted in marked elevations in osmolar excretion and in solute-free water reabsorption. However, no change in plasma VP concentration was observed even after the largest dose of AA which raised their plasma concentration from 3.0 to 7.4 mmol/L. At first sight, this result suggests that AA did not influence VP secretion.

Alternatively, the rise in PVP due to AA changes may have been masked by a simultaneous negative influence. It is probably the case because a significant dose-dependent fall in PNa was observed, and PNa is known to be the most efficient plasma solute influencing VP secretion [20]. In contrast, urea is known to have little effect because it freely equilibrates in extra and intracellular compartments and thus does not represent an “effective” solute for osmotic sensor cells in the neurohypothalamus [20]. As shown in figure 5, the “effective” osmotic pressure did not change during AA infusion, as a result of opposite changes in PAA and PNa. Should no other osmole had interfered, the fall in PNa would have induced a fall in PVP [38]. Thus, we may reasonably propose that the lack of change in plasma VP in the present study results from a balance between two opposite influences on VP secretion, one, negative, due to the significant fall in PNa, and one positive, due to the rise in PAA. Accordingly,
AAs represent an effective osmotic stimulus for VP secretion which is almost equally potent as that induced by sodium.

Why did PNa drop during AA infusion without an associated change in plasma chloride? Plasma AA plateaued after one hour although the infusion was maintained for three hours. Thus, a large amount of AA must have been stored or partly metabolized in the liver as indicated by the rise in plasma urea and in urea excretion. Cellular uptake of AA is achieved through sodium-coupled cotransporters. Thus, the fall in PNa most probably results from a simultaneous cellular uptake of AAs and sodium.

It may be surprising to observe a dose-dependent increase in urinary VP excretion in response to AA infusion, in the absence of any change in PVP. However, previous studies have well established that urinary VP excretion may be influenced by other factors than PVP. First, the rise in GFR induced by AA infusion should increase proportionately the renal clearance of VP, explaining about 1/5 of the rise in urinary VP seen in the High-AA test. Second, as well documented in several different experimental and pathological situations, changes in osmolar excretion markedly influence VP handling by the renal tubule and thus VP recovery in the urine, independently of any change in PVP and GFR [45]. In rats with DM (a condition in which solute excretion rises dramatically due to glycosuria), urinary VP excretion was found to increase more than fifteen-fold when PVP rose only three-fold [46]. Thus, the marked rise in osmolar excretion which occurred after AA infusion may have reduced VP re-absorption proportionately, as suggested by parallel changes in VP and osmole excretions (Fig 3).

The primary aim of this study was to determine if a disturbance in plasma AA could account for the elevated VP secretion reported in diabetes mellitus, a chronic disorder. This study explored the influence of an acute change in AAs
Figure 3
Time course of changes in VP excretion (top), osmolar excretion (middle), and solute-free water reabsorption ($T^{H_2O}$) (bottom) during the three tests. Symbols and abscissa as in Figure 2. See Table I for statistical analysis.
which led to abrupt changes in other plasma solutes including sodium. A significant decrease in $P_{Na}$ also occurs during chronic hyperglycemia [1, 5, 18, 19, 47, 48]. However, during a chronic situation with permanent elevations of glucose and AAs, a new steady state is established in which various regulatory adaptations “reset” the threshold for VP secretion to a lower level in relation to the actual $P_{Na}$. This may give more importance to AAs in inducing a rise in VP secretion. The same applies for the rise in plasma glucose. Zerbe et al. concluded that glucose did not participate in the rise in PVP seen in diabetes mellitus because they observed no rise in PVP after an acute infusion of hypertonic dextrose in healthy subjects or in patients with DM [18]. However, and similarly to what was observed in the present study, $P_{Na}$ fell during these acute dextrose loads. Accordingly, both glucose and AA could contribute to stimulate VP secretion in DM, although this effect is masked after acute loads because of the counteracting effect of a simultaneous acute decline in $P_{Na}$.

Do all AAs influence VP secretion equally? Although the setting of this experiment does not allow us to answer this question, it may be assumed that branched chain AAs have the most significant influence on VP secretion. Indeed, their plasma concentration after a protein meal is known to remain elevated for a longer duration than that of other AAs [49], probably because they undergo little degradation in the liver. Leucine and valine are the two most abundant branched chain AA in plasma. The level of these two AAs rose quite significantly in the present study, and in diabetes mellitus their plasma level is more enhanced than that of other AAs [26, 27].

In conclusion, the present results suggest that AAs behave as osmotically active solutes which can stimulate VP secretion. However, in acute experiments, the concomitant fall in plasma sodium (likely due to intense cotransport with AA) tends to reduce VP secretion and to hamper the expected rise induced by AAs. Whether an increase in
References