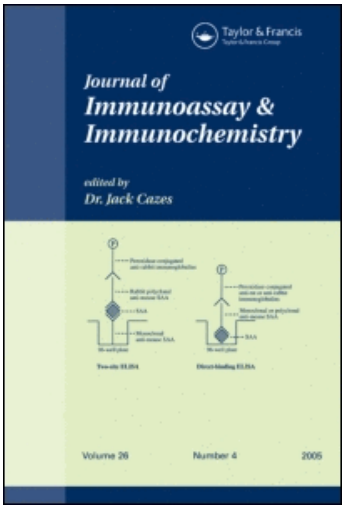

Journal of Immunoassay and Immunochemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597271

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To cite this Article Smitz, Simon , Legros, Jean-Jacques and Maire, Marc le(1996) 'Evidence for Circulating Vasopressin-Like Peptides in a Case of Polyuria', Journal of Immunoassay and Immunochemistry, 17: 3, 227 — 243
To link to this Article: DOI: 10.1080/01971529608005790
URL: http://dx.doi.org/10.1080/01971529608005790

JOURNAL OF IMMUNOASSAY, 17(3), 227-243 (1996)

EVIDENCE FOR CIRCULATING VASOPRESSIN-LIKE PEPTIDES IN A CASE OF POLYURIA

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ABSTRACT

Plasma immunoreactive vasopressin (iAVP) was studied by RIA in a patient suffering from polyuria during chronic treatment with lithium. The combined use of two antisera specific for different regions of the AVP molecule allowed us to detect circulating forms which are modified in the acyclic tripeptide portion.

In this lithium-treated patient, iAVP was abnormally low with respect to plasma osmolality. However, iAVP increased during hypertonic saline infusion, probably through an osmosensitive mechanism. A remarkable finding was that contrary to the observations made in healthy subjects and in another patient with diabetes insipidus, iAVP measured with the antiserum specific for the acyclic portion of the AVP molecule was below the values measured with the antiserum specific for the hexapeptide ring. This unusual immunoreactivity profile suggests that the plasma of this polyuric lithium-treated patient contains vasopressin-like peptides which differ from arginine vasopressin in the structure of the C-terminal tripeptide tail.

(KEY WORDS : vasopressin, vasopressin-like, polyuria, diabetes insipidus, immunoassay, lithium)

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INTRODUCTION

Lithium, which is used in the treatment of manic-depressive disorders, can influence both the secretion and action of vasopressin (1-6). Direct measurements of plasma vasopressin by RIAs have shown that the polyuria observed in certain patients treated with lithium was most often caused by nephrogenic diabetes insipidus. However, a partial cranial diabetes insipidus has been identified in one patient (4).

We have observed a patient who had polyuria during chronic treatment with lithium. In order to determine the neuro-endocrine abnormalities responsible for this polyuria, a quantitative and qualitative study of circulating vasopressin was performed. The characterization of the circulating vasopressin was carried out by RIAs using two antisera highly specific for different regions of the arginine-8-vasopressin molecule (AVP).

MATERIALS AND METHODS

Subjects and Patients

1. Lithium-treated patient : A 55-year-old woman was referred to our outpatient service for evaluation of polyuria. This patient had been treated with lithium for 14 years because of depressive disorders. A hypothyroidism had been corrected for 6 years by the administration of L-thyroxin. Polyuria had been observed for several years (diuresis above 2.5 liters/day). This marked polyuria had caused the cessation of lithium treatment. Urea, creatinine and serum ions

were within the reference interval. Urine osmolality fluctuated between 153 and 250 mmol/kg.

Plasma immunoreactive vasopressin (iAVP) and osmolality (plasma and urine) were measured in this patient as an outpatient after the lithium had been withheld for more than 10 days. Because of the unusual immunoreactive profile (Table 1), a hypertonic saline infusion (HSI) was performed three weeks later.

Three years after the performance of the HSI reported in this study, the patient was rehospitalized for treatment of depressive disorders. During this hospitalization, since the water intake was spontaneously low, the patient developed consciousness disorders associated with the perturbation of the water metabolism : serum Na^+ = 169 mmol/L, K^+ = 4.7 mmol/L, total serum calcium 2.35 mmol/L, urea 15 mmol/L, plasma osmolality = 348 mmol/kg. Urine osmolality fluctuated between 199 and 261 mmol/kg. A tomodensitometric examination of the encephalon showed a left posterior hemorrhagic softening and a right thalamo-capsular zone of ischemia. A CT scan centered on the sella turcica was normal.

During the following 4 years, hypernatremia (serum Na^+ 148-161 mmol/L) was observed and three serious dehydration episodes, due to the chronic hypodipsia, required hospitalization. Seven years after the HSI, the patient was readmitted for severe dehydration. Three months earlier, a brain scan showed the persistence of a small right thalamic zone of ischemia and the sella turcica was normal. On admission the serum sodium was 161 mmol/L, potassium 4 mmol/L,

TABLE 1

Determination of Plasma Osmolality, Plasma Immunoreactive Vasopressin (iAVPAS1 and iAVPAs2) in Normal Subjects and in Patients with Diabetes Insipidus. Calculation of the Ratio iAVPAs1/iAVPAs2.

	Plasma osmolality (mmol/kg)	iAVPAs1 (ng/L)	iAVPAs2 (ng/L)	Ratio iAVPAs1 / iAVPAs2
Normal Subjects (n = 12)	284.1 ± 2.7	2.6 ± 1.3	5.8 ± 1.8	0.44 ± 0.13
Patient P1 (NEDI)				
basal	287	4.7	34.0	0.14
10 min after apomorphine 0.5 mg SC		2.5	17.5	0.14
25 min after apomorphine (nausea)		115	142	0.81
Patient P2 (CDI)				
before HSI	286	0.8	-	-
end of HSI	306	1.5	< 2.0	NC (> 0.75)
60 min after dDAVP (5 µg IM)	296	45	< 2.0	NC (> 22.5)
Patient P3 (CDI)				
before HSI	289	1.6	-	-
end of HSI	299	2.0	1.9	1.05
Patient P4 (CDI)	341	0.5	-	-
Patient P5 (CDI)				
before HSI	291	2.0	< 4.0	NC (> 0.5)
end of HSI	302	2.4	< 4.0	NC (> 0.6)
60 min after dDAVP (5 µg IM)	291	64	< 4.0	NC (> 16)
Lithium-treated Patient				
basal (outpatient)	294	4.5	1.8	2.5
before HSI (- 10 min)	308	3.1	< 2.0	NC (> 1.5)
before HSI (0 min)	307	2.7	2.5	1.1
during HSI test (n = 7)				2.15 ± 0.94
end of HSI	343	74.7	42.0	1.8

NEDI : nephrogenic diabetes insipidus
CDI : cranial diabetes insipidus
HSI : hypertonic saline infusion
P4 : cerebral hemorrhage, comatose
P1 : Bartter's syndrome
P2 : craniopharyngioma
P3 and P5 : head trauma (dDAVP withheld 20-24 h before HSI)
NC : not calculated (iAVPAs2 value being below the detection limit)

chloride 118 mmol/L, plasma osmolality 345 mmol/kg, plasma vasopressin-neurophysin 0.3 μg/L, urea 36 mmol/L and urine osmolality 330 mmol/kg. Eleven days later, at the time of discharge, serum sodium (144 mmol/L) and urea (6 mmol/L) were normalized.

2. Normal subjects and other patients : The results of iAVP determinations in 12 healthy subjects have been previously reported (7). Plasma vasopressin was also studied in five patients (P1 to P5) with polyuria (Table 1). These patients have never been treated with lithium. In patient P2, a craniopharyngioma had manifested itself and was operated 6 years after the evaluation of the hypothalamo-neurohypophyseal function. In patient P3, polyuria appeared 8 days after cranial trauma. Treatment with dDAVP (Minrin, Ferring) one or two times 5 μg/day (intranasal) had been in place for 3 years. In patient P4, the determinations were performed one day after a cerebro-meningeal hemorrhage. Patient P5 developed polyuria 3 days after cranial trauma and had been treated with intranasal dDAVP for the 7 weeks preceding the HSI.

All subjects and patients gave their informed consent prior to the studies.

Experiments

Unless indicated, the subjects and patients were fasting (water intake was however authorized) and abstained from smoking, drinking alcoholic beverages and/or taking drugs on the day of the study.

Plasma osmolality and vasopressin were measured in the lithium-treated patient 1) under basal conditions (out-patient, without water restriction)

2) three weeks later, during a HSI. Excess fluid intake was avoided during the 10 hours preceding the HSI by mild water restriction.

In the patients as well as in one healthy subject, HSI was carried out in a recumbent position by perfusing 5% NaCl into an antecubital vein at 0.06 ml/kg/min for 120 minutes. A pump was used (constant infusion rate of hypertonic saline) to achieve a smooth increase in plasma osmolality. In patient P3 after 40 minutes of perfusion the feeling of thirst was intense, therefore the flow rate was brought to 0.03 ml/kg/min and 400 ml of water were absorbed. Two blood samples were taken before perfusion (-10 and 0 minutes), then every 20 minutes during HSI.

Radioimmunoassays and Extraction

The treatment of the plasma samples and the osmolality measurements (performed 2-3 hours after HSI) were carried out as described previously. The plasma samples were stored at -20° C for extraction within two weeks and assay within 3 weeks. iAVP was determined by RIA using two antisera : As1 and As2 (7,8). Briefly, antiserum As1 is highly specific for the hexapeptide ring of AVP while antiserum As2 is specifically directed towards the antigenic determinants of the amino acid residues at positions 8 and 9 (acyclic portion of AVP). In healthy subjects, iAVP measured with antiserum As1 (iAVPAs1) is lower than the immunoreactivity measured using antiserum As2 (iAVPAs2), the ratio of iAVPAs1/iAVPAs2 being 0.44 ± 0.13 (Table 1).

We also observed that the immunoreactivity measured using As1 was lower than the immunoreactivity measured using As2 when the plasma extracted components were separated by Sephadex G25 chromatography. This difference was due to the fact that As2, but not As1, detected a material which was eluted before the elution volume of AVP (Smitz, unpublished results). These results confirm the observations made by other authors (9) and can be explained by the fact that the antisera specific for the C-terminal tail can detect certain interfering molecules, specially degradation products of AVP. Measurements of plasma vasopressin-neurophysin were performed by RIA according to the technique described (10).

The results are expressed as the mean ± SD. The statistical significance of the results was evaluated using the Student t test. The results are not corrected for loss during the extraction procedure.

RESULTS

In a healthy subject, an increase in plasma osmolality (from 283 to 294 mmol/kg) and iAVP were observed during the HSI (Fig. 1). During this test, the ratio iAVPAs1/iAVPAs2 was 0.56 ± 0.10 (n=9).

In the lithium-treated patient :

1. in the basal conditions, the plasma osmolality was 294 mmol//kg, urine osmolality 245 mmol/kg, iAVPAs1 4.5 ng/L, iAVPAs2 1.8 ng/L with an iAVPAs1/iAVPAs2 ratio equal to 2.5, a value significantly higher ($p < 0.01$)

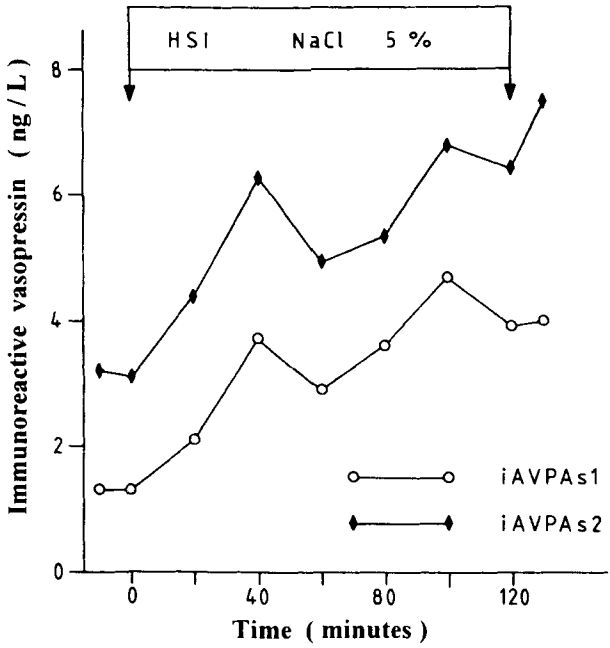


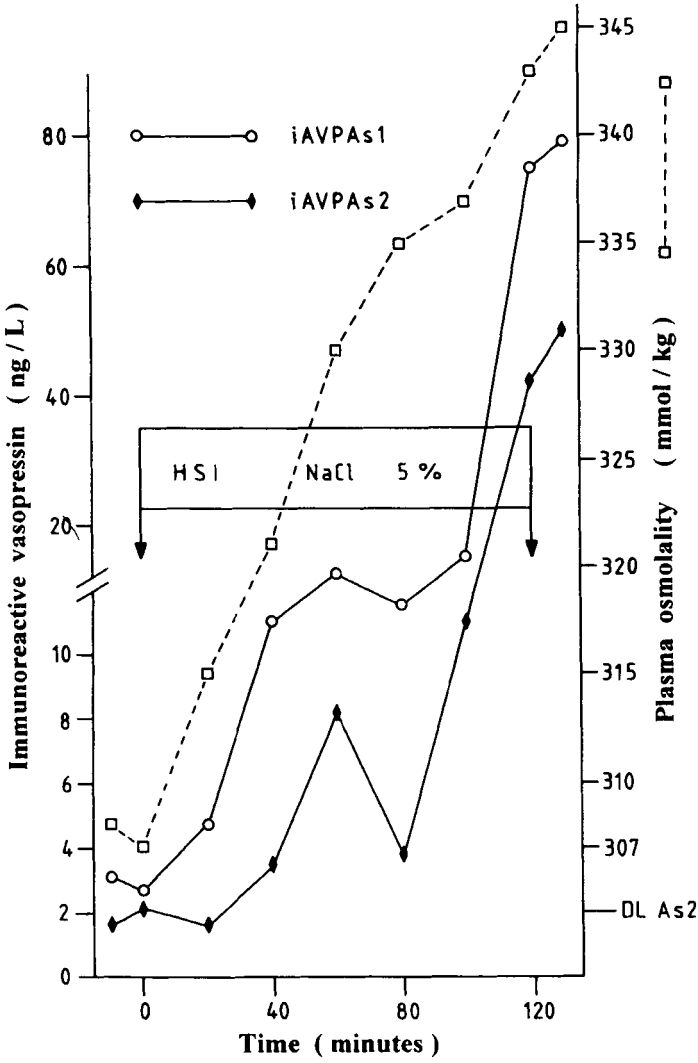
FIGURE 1. Course of the plasma immunoreactive vasopressin (iAVP) concentrations during hypertonic saline infusion (HSI) in a normal subject. Infusion of 5 % saline into an antecubital vein was performed at the rate of 0.06 ml/kg/min for 120 minutes. iAVP was measured by antiserum As1 (iAVPAs1 O———O) and by antiserum As2 (iAVPAs2 ♦———♦). During this test, an increase in plasma osmolality from 283 to 294 mmol/kg was observed and the ratio iAVPAs1/iAVPAs2 was 0.56 ± 0.10 (n = 9). As1 is specific for the hexapeptide ring of AVP while As2 is specifically directed towards the acyclic tripeptide portion of AVP.

than the values obtained with the 12 healthy subjects (Table 1).

2. after a moderate water restriction period (10 minutes before and just before HSI), plasma osmolalities were 308 and 307 mmol/kg respectively, urine being hypotonic relative to plasma (250 mmol/kg) and the sensation of thirst was present albeit weak. iAVPAs1 values were 3.1 and 2.7 ng/L respectively. The corresponding iAVPAs2 values were lower : below the detection limit (2.0 ng/L in this case) and 2.5 ng/L, respectively.
3. during HSI plasma osmolality increased from 307 mmol/kg to 343 mmol/kg, iAVPAs1 increased from 2.7 ng/L to 74.7 ng/L and iAVPAs2 from 2.5 ng/L to 42 ng/L (Fig. 2). Ten minutes after the HSI, 345 mmol/kg, 79.3 ng/L and 45.7 ng/L were the values obtained for plasma osmolality, iAVPAs1 and iAVPAs2 respectively. The sensation of thirst was present during HSI (and was the most intense at 20-25 min) but with low intensity relative to the degree of plasma hyperosmolality. During this test, the ratio iAVPAs1/iAVPAs2 was 2.15 ± 0.94 (n=7) a significantly higher value ($p<0.01$) than the value observed with healthy subjects (Table 1). It should also be noted that iAVPAs2 only went above 2 ng/L when the plasma osmolality was more than 315 mmol/kg (Fig. 2).

DISCUSSION

The antisera As1 and As2 are specific for the cyclic and acyclic regions of arginine vasopressin respectively (7). The simultaneous use of both these antisera



will allow the detection of circulating vasopressin forms whose acyclic portion has been modified.

In the lithium-treated patient :

1. the plasma osmolality value before HSI was unusually high (308 mmol/kg). In four other conscious patients with diabetes insipidus, the values were between 286 and 291 mmol/kg (Table 1). This unexpected hyperosmolar state might have been precipitated by the lithium withdrawal since it is well recognized that lithium can stimulate thirst in humans.

2. the large increase in plasma osmolality during HSI was also unusual (and involuntary, osmolality not being monitored during the test) : from 308 to 343 mmol/kg (5.7 % / hour). By comparison, in the three patients with CDI, plasma osmolality increased from 286 to 306 mmol/kg, 289 to 299 mmol/kg and from 291 to 302 mmol/kg (Table 1). Large increases in plasma osmolality have also been observed during HSI in several polyuric patients with central diabetes

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FIGURE 2. Effect of infusion of hypertonic saline (HSI) on plasma immunoreactive vasopressin (iAVP) and plasma osmolality (□-------□) in the patient with polyuria during lithium treatment. Infusion of 5 % saline was performed at the rate of 0.06 ml/kg/min for 120 minutes. iAVP was measured by antiserum As1 (iAVPAs1 O——O) and by antiserum As2 (iAVPAs2 ♦———●). The detection limit of the assay for plasma iAVPAs2 is indicated by DLAs2. Note that in contrast to the normal subject (see Fig. 1), iAVPAs1 was higher than iAVPAs2. As1 is specific for the ring and As2 for the tripeptide tail of arginine vasopressin.

insipidus (11,12). The mild fluid restriction and a decrease in the sensation of thirst favored this unusual increase in plasma osmolality.

3. plasma vasopressin was abnormally low relative to plasma osmolality since iAVPAs2 only went above 2 ng/L when plasma osmolality was above 315 mmol/kg. This vasopressin deficit, characteristic of a cranial diabetes insipidus, was probably more severe since iAVPAs2 does not only detect AVP, but also the degradation products of this hormone. Vasopressin secretion increased nonetheless, when the plasma was highly hypertonic. Since no nonosmotic stimuli has been observed, this increase takes place on the intervention of an osmosensitive mechanism. Seven years later, during severe dehydration, plasma vasopressin-neurophysin remained low despite hyperosmolality and hypovolemia. Since vasopressin-neurophysin is released in response to stimuli known to release vasopressin, this low neurophysin level may also reflect partial central diabetes insipidus. These data and the natural evolution (chronic hypernatremia) suggest a severe dysfunction of the osmoregulatory system in this lithium-treated patient. Chronic hypernatremia due to a markedly elevated threshold for vasopressin release and thirst has been described in a patient who had received prolonged lithium treatment (12). The cause of the reset of osmostats which persisted in this patient despite stopping the lithium therapy for one year remained speculative (12).

4. A remarkable finding was the unusual immunoreactivity profile. The latter does not seem to be related to an artifact since it is observed both on the basal

state and when iAVP increases under the effect of the osmotic challenge. This increase in the iAVPAs1/iAVPAs2 ratio was not found a) in another patient presenting with diabetes insipidus (P1, Table 1) b) in patients with different pathological conditions (excluding a patient with SIADH and an oat cell carcinoma, see below) even when these pathological conditions involved a neurohypophyseal stimulation (7). Since vasopressin concentrations in some patients with cranial diabetes insipidus were very low (below the detection limit, see Table 1), the iAVPAs1/iAVPAs2 ratio of these patients was not available for comparison with that of the lithium treated patient. As we have observed after administration of DGAVP (13) and dDVAP (Table 1), a higher iAVPAs1/iAVPAs2 ratio strongly suggest the presence of circulating vasopressin-like peptides that are distinguished from AVP by the nature or conformation of their acyclic portion.

In certain patients who presented with polyuria during lithium treatment, a vasopressin deficit was suspected (1,6) and confirmed by the direct determination of plasma vasopressin (4). To the best of our knowledge, this study is the first attempt aiming to specify the nature of the circulating vasopressin in a case of polyuria after prolonged lithium administration. Due to the limited amount of serum, we have not performed any chromatography. For this reason, we know neither the number, nor the proportion of the circulating vasopressin forms. However, these vasopressin like-peptides, which are extracted from the plasma by our method, in all likelihood have a molecular weight close to that of AVP since

1) the cross-reactivity of As1 with the higher molecular weight form of vasopressin is low (7,14)

2) the recovery of higher molecular weight forms after extraction is low.

These forms could have lost their antidiuretic activity since the residues at positions 8 and 9 are implicated in the antidiuretic activity (15,16). The secretion of vasopressin-like peptides could result from the synthesis of a modified mRNA and/or perturbations in the processing of the AVP precursor. Interestingly, circulating AVP analogues which antagonize the action of AVP have been suspected in early diabetes insipidus after hypothalamic surgery (17). In a previous work, the heterogeneity of circulating vasopressin was demonstrated in a patient presenting a SIADH associated with a lung tumor (7). The iAVPAs1/iAVPAs2 ratio observed in this patient also suggested the presence of peptides similar to vasopressin but differing from the latter in the structure of the C-terminal tail. In the lithium-treated patient, no lung tumor nor SIADH were detected. On the contrary, the patient presented a hypernatremia syndrome with adipsia. Even though the lesions confirmed by the brain scans were not situated in the hypothalamo-neurohypophyseal region, the causes (dehydration) and consequences of these lesions remain uncertain. Since lithium can interfere with the synthesis, storage and/or central release of vasopressin (2), it is likely that this drug has favored the neuroendocrine abnormalities and severe osmoregulation disorders that have been observed. We cannot, however, exclude an effect due to other drugs since many other drugs were also administered to this patient.

Immunoassay study of circulating vasopressin forms has given a fresh impetus to research into the mechanisms involved in the pathogenesis of familial neurogenic diabetes insipidus. As pointed out by Robertson (18), this approach has « ... made it possible for the first time to establish unequivocally the natural history of the AVP deficiency in this disorder ... ». Further studies are needed to determine the usefulness of the approach developed here and its contribution to the understanding of certain cases of acquired diabetes insipidus.

ACKNOWLEDGMENTS

We would like to thank the physicians who allowed us to study their patients. We are indebted to the excellent nursing staff of the Metabolic Unit of the CHU and to Mrs L.M. Olivera, Mrs M. Fodor, Mrs M. Delbouille, Dr. V. Fraipont and Mr M. Matthys for assistance in preparing this manuscript. The first author wishes to thank Mr and Mrs J. Smitz-Mouchomorine (SMLab) for care of the animals.

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