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Erratum

Unfortunately the paper by Gustawa Stendig-Lindberg et al. printed in this j. 29 (1991) (12) 833–836 contains the following errors:

It was prepared for the 1992 volume but published in December 1991. The citation (top left-hand corner, p. 833) should therefore read:
Eur. J. Clin. Chem. Clin. Biochem.
Vol. 29, 1991, pp. 833–836

In table 1, p. 834, the range for serum magnesium should read:
0.72–0.91 (mmol/l).

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A Highly Sensitive and Rapid Radioimmunoassay for the Determination of Arginine[8]-Vasopressin

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Summary: A highly sensitive radioimmunoassay for arginine[8]-vasopressin (argipressin; INN) measurement was developed using Amberlite XAD 2 resin columns to extract arginine[8]-vasopressin from acidified human plasma. Arginine[8]-vasopressin was determined by a rapid radioimmunoassay method (2 × 20 h) using a specific antibody and ^{125}I-labelled antigen. The bound fraction was separated by adsorption of the free fraction onto bovine serum albumin-coated charcoal; this resulted in low unspecific binding of less than 2%. Recovery experiments in the physiological range resulted in a mean (± SEM) recovery of 88 ± 3%. The radioimmunoassay consistently yielded a detection limit of 0.3 ng/l (ED_{90}) and a mean 50% binding intercept (ED_{50}) of 3.5 ng/l. Arginine[8]-vasopressin immunoreactivity was characterized by reverse-phase high performance liquid chromatography, which confirmed the specificity of the assay. Serial plasma dilution curves paralleled the standard curve. The intra- and inter-assay variations were 9.4% and 15%, respectively. Arginine[8]-vasopressin concentrations in healthy subjects were determined in normal hydration status (2.2 ± 0.3 ng/l; n = 11), as well as during suppression by water immersion (1.5 ± 0.2 ng/l; n = 11) or by water loading (1.6 ± 0.2 ng/l; n = 8). Thus, this assay allows for a sensitive, accurate and rapid quantification of plasma arginine[8]-vasopressin concentrations.

Introduction

Alterations of arginine[8]-vasopressin plasma concentrations seem to be involved in the abnormal water handling that is present in several diseases. Thus, investigations in patients with congestive heart failure (1–3), hyperaldosteronism (4) or syndrome of inappropriate antidiuresis (5, 6) demonstrated abnormal arginine[8]-vasopressin secretion as a determinant of impaired water balance. Furthermore, any investigation of the physiological regulation of water homeostasis should comprise determination of arginine[8]-vasopressin plasma concentrations (7, 8). Therefore, an accurate determination of arginine[8]-vasopressin is important for both physiological and clinical purposes. Efforts to obtain an exact measurement of the rather low plasma concentrations of arginine[8]-vasopressin by radioimmunoassay have been hampered by the presence of different interfering factors which are still only partially characterized. Thus, limited specificity is inherent in the methods using direct radioimmunoassay for arginine[8]-vasopressin in plasma (9, 10), necessitating plasma extraction before radioimmunoassay of the neurohypophyseal peptide. Different extraction procedures have been used, including the use of acetone and petroleum ether (11, 12), cold ethanol (13) or Bentonite (14), but they show poor and variable recoveries (11, 13). Furthermore, several radioimmunoassays for arginine[8]-vasopressin involve complicated separation techniques, and in order to obtain adequate sensitivity they employ incubation periods of up to 7 days (9, 15).

Therefore we have developed a simple, accurate and rapid method for determining arginine[8]-vasopressin following extraction from plasma. The method has a very low detection limit.

Materials and Methods

Material

Sample preparation and extraction

Blood was collected in disodium ethylenediaminetetraacetate coated tubes and immediately centrifuged at 2000 *g* for 10 min at 4 °C. The plasma was removed, treated with phenylmethylsulphonylfluoride (5 mmol/l) and acidified with acetic acid to pH 5.5 (2.5 mol/l). For extraction the plasma samples were adsorbed as 1 ml aliquots to activated Amberlite XAD 2 resin (Serva, Heidelberg, Germany) chromatography columns (Bio-Rad Laboratories, Richmond, CA 94804) followed by washing with trifluoroacetic acid (1 mmol/l). The elution was performed using a mobile phase consisting of 1 mol/l acetonitrile (80%) and 1 mmol/l trifluoroacetic acid (20%). The lyophilized samples were reconstituted in 300 µl assay buffer I.

Recovery

Recovery experiments were performed by parallel extraction of 1, 2, 4 and 8 pg synthetic arginine[8]-vasopressin (Bissendorf Biochemicals GmbH, Hannover, Germany) added to 1 ml aliquots of different plasma samples.

Assay buffers

Two assay buffers were used in this radioimmunoassay.

Assay buffer I was prepared with 20 g/l of bovine serum albumin in 50 mmol/l phosphate buffer (pH 7.4) containing 5 mmol/l phenylmethylsulphonylfluoride, 0.1 mol/l sodium chloride, 10 mmol/l disodium ethylenediaminetetraacetate and 0.1 mol/l Triton X-100. After incubation for 4 h at 56 °C the buffer was diluted 10 fold. Neomycin sulphate (2 g/l) and sodium azide (1 g/l) were then added and the pH adjusted to 7.4.

For assay buffer II dissolved gelatine (1 g/l) was added to 20 mmol/l phosphate buffer containing 0.15 mol/l sodium chloride, 0.1 g/l bovine serum albumin and 0.1 g/l thimerosal. Thereafter the pH was adjusted with sodium hydroxide (10 mol/l) to 7.4.

Standard curve

Known amounts of synthetic arginine[8]-vasopressin were serially diluted ranging from 80 to 0.08 ng/l in assay buffer I.

Methods

Assay protocol

Polyclonal antibody (Nol 728-6), generated against synthetic arginine[8]-vasopressin bovine thyroglobulin conjugates in rabbits (16), was diluted to bind about a third of the total radiolabelled antigen added in the absence of unlabelled antigen. Antiserum (100 µl, end dilution 1 : 50 000) was added to standards and extracted antigens. Incubation was carried out in polypropylene tubes for 20 h at 4 °C. Then, 1.6 pg/tube ^{125}I-labelled arginine[8]-vasopressin (Amersham Braunschweig, Germany, 74 TBq/mmol (2000 Ci/mmol); 100 µl) was added, followed by incubation at 4 °C for a further 20 h.

In order to determine cross-reactivities of the antibody with other peptides, standard curves of the radioimmunoassay were performed with ocytocin, atrial natriuretic peptide, angiotensin II and lysine[8]-vasopressin, respectively, instead of unlabelled arginine[8]-vasopressin.

Separation

Separation was performed by adding 500 µl of bovine serum albumin-coated charcoal (29%/71%, by weight) dissolved in assay buffer II. After 10 min, standards and samples were centrifuged at 4 °C for 10 min at 2000 *g* and the supernatant was counted (LKB, Turku, Finland, 80% efficiency) in triplicate.

Non-specific binding was determined by performing the radioimmunoassay without antibody (n = 9).

Reverse-phase-HPLC

Arginine[8]-vasopressin immunoreactivity was characterized by reverse-phase high performance liquid chromatography (n = 6). For calibration a standard of 200 pg synthetic arginine[8]-vasopressin was dissolved in 250 µl acetonitrile and submitted to reverse-phase high performance liquid chromatography (C_8-ultrapore TM column [5 µm], Beckman San Ramon, U.S.A.). Standard and extracted samples were eluted with a linear gradient from 15% – 45% acetonitrile in 1 mmol/l trifluoroacetic acid in 35 min (flow rate 0.5 ml/min, fraction size 0.5 ml/tube). Subsequently, the hormone contents of the fractions were measured by radioimmunoassay.

Water immersion

Water immersion was performed as described in detail elsewhere (17–19). Briefly, following 1 h in the sitting position at room temperature (28 °C) beside the water thank, 11 healthy, normally hydrated subjects were immersed to the level of the neck into thermoneutral water (34.5 °C) for 1 h followed by 1 h outside the tank. Blood samples were taken immediately before, after 1 h immersion and 1 h after exiting the tank.

Water loading

After an overnight fast (16 h) blood samples (basal value) were taken from 8 healthy volunteers. Thereafter the subjects were orally water loaded with 20 ml/kg body weight within 30 min and blood samples were taken 1 h later.

All data are given as mean ± standard error of the mean. Significance of differences was tested by the *Student* t-test.

Results

Recovery experiments (n = 11) with physiological concentrations of synthetic arginine[8]-vasopressin added to different plasma samples prior to extraction showed a linear relationship between added and determined arginine[8]-vasopressin concentration with a mean recovery of 88 ± 3%. Figure 1 illustrates a typical standard curve for the radioimmunoassay of arginine[8]-vasopressin with the 50% binding intercept (ED_{50}) at 3.5 ng/l and a lower detection limit (ED_{90}) of 0.3 ng/l (n = 8). Serial dilutions of 0.5, 1, 2 and 4 ml plasma paralleled the standard curve. The intra-assay coefficient of variation (n = 8) averaged 9.4%, the inter-assay coefficient of variation (n = 8) was

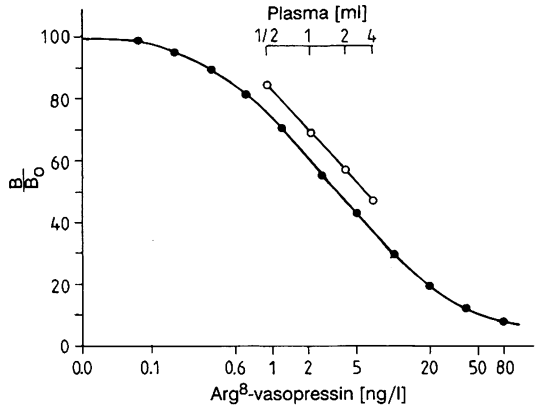

Fig. 1. Typical standard curve with a low detection limit (ED_{90} = 0.3 ng/l, ED_{50} = 3.5 ng/l and ED_{10} = 58.1 ng/l). Serial dilutions of plasma paralleled the standard curve, confirming the accurate measurement of plasma samples.

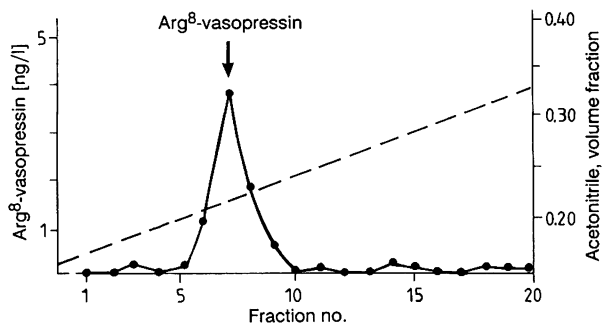

Fig. 2. Coelution of arginine[8]-vasopressin immunoreactivity of an extracted plasma sample with synthetic arginine[8]-vasopressin by reverse-phase high performance liquid chromatography analysis, confirming the specificity of the assay.

15%. No cross-reactivity of the anti-arginine[8]-vasopressin antibody to ocytocin, atrial natriuretic peptide and angiotensin II could be observed. The cross-reactivity to lysine[8]-vasopressin was 3%. The non-specific binding was less than 2%.

Reverse-phase high performance liquid chromatography analysis revealed coelution of immunoreactive arginine[8]-vasopressin with synthetic arginine[8]-vasopressin as a single peak (fig. 2).

Central volume stimulation by 1 h head-out water immersion in 11 healthy subjects produced a significant drop (2.2 ± 0.3 vs. 1.5 ± 0.2 ng/l, $p < 0.05$) of arginine[8]-vasopressin plasma concentrations. In the recovery period, arginine[8]-vasopressin returned to baseline concentrations (1.5 ± 0.2 vs. 2.0 ± 0.3 ng/l, $p < 0.05$). Oral water loading of 8 healthy subjects significantly decreased arginine[8]-vasopressin plasma concentrations (2.0 ± 0.2 vs. 1.6 ± 0.2 ng/l, $p < 0.05$) as well as plasma osmolality (282 ± 5.5 vs. 274 ± 4.4 mosmol/kg, $p < 0.05$). Arginine[8]-vasopressin plasma concentrations were significantly correlated with plasma osmolality ($r = 0.83$, $p < 0.01$).

Discussion

Due to the very low plasma concentration of arginine[8]-vasopressin and the presence of inadequately characterized interfering factors, the development of a sensitive and specific radioimmunoassay for arginine[8]-vasopressin has proved to be more difficult than for many other peptides (9).

In contrast to a variety of less specific antisera raised for vasopressin determination (20–23), we used a highly specific one (16). Due to the specificity of this antibody (confirmed by cross-reactivity experiments and high performance liquid chromatography analysis), the extraction procedure was simpler than those reported previously for other methods. The usefulness of this Amberlite XAD 2 resin extraction procedure was confirmed by the linear relationship between added and measured arginine[8]-vasopressin concentration in the recovery experiments, as well as by a reliable mean recovery rate of 88%.

Tab. 1. Arginine[8]-vasopressin assays described by different authors

Author	Lower detection limit	ED_{50}	Mean recovery	Incubation time	HPLC analysis
Bichet, D. G. (24)	0.5 ng/l	1.2 pg/tube*	102%	120 h	+
Bie, P. (25)	0.5 pg/tube*	8.5 pg/tube*	72%	?	–
Bodola, F. (26)	0.4 ng/l	4.6 ng/l	80%	32 h	–
Durr, J. A. (27)	0.2 pg/tube*	5.0 pg/tube*	103%	96 h	–
Fyhrquist, F. (10)	1.2 ng/l	8.3 ng/l	96%	168 h	–
Gerbes, A. L. (this paper)	0.3 ng/l	3.5 ng/l	88%	40 h	+
Husain, M. K. (11)	0.1 ng/l	0.8 ng/l	68%	120 h	–
Keil, L. C. (28)	1.6 ng/l	4.2 pg/tube*	64%	134 h	–
Robertson, G. L. (9)	0.5 ng/l	1.1 pg/tube*	96%	168 h	–
Ross, M. G. (29)	0.8 ng/l	4.0 ng/l	70%	?	–
Samson, W. K. (16)	1.0 pg/tube*	25.0 pg/tube*	80%	96 h	–
Skowsky, W. R. (14)	0.7 ng/l	10.8 ng/l	84%	120 h	

* where absolute concentrations could not be derived, data are adopted as specified in the cited publication

The radioimmunoassay consistently yielded a detection limit of 0.3 ng/l after only 2 × 20 h of incubation. In other methods, much longer incubation times are needed to obtain a comparably low detection limit (tab. 1). The present assay can therefore be considered as both highly sensitive, and more rapid than other assays.

Arginine[8]-vasopressin immunoreactivity determined in plasma was characterized by reverse-phase high performance liquid chromatography. Immunoreactive arginine[8]-vasopressin was coeluted with synthetic arginine[8]-vasopressin as a single peak, confirming the specificity of the assay.

Isoosmotic arginine[8]-vasopressin suppression by central volume stimulation during water immersion (30, 31) was clearly demonstrated in accordance with the findings of other groups, supporting the concept of inhibition of arginine[8]-vasopressin secretion by acute atrial distension (32, 33).

Hypoosmolality as an osmotic stimulus of arginine[8]-vasopressin suppression (9) was confirmed by a significant decrease of plasma arginine[8]-vasopressin concentrations and plasma osmolality after oral water loading. Both quantities were closely correlated.

In conclusion, this radioimmunoassay for arginine[8]-vasopressin can be considered as highly sensitive, accurate and rapid, and it can be easily established in any suitably equipped laboratory.

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