Biological half-life and organ distribution of $[^3]$H$8$-arginine-vasopressin in the rat

T. Janáky, F. A. László, F. Sirokmán and J.-L. Morgat†

Endocrine Unit and Research Laboratory, First Department of Medicine, University Medical School, Szeged, Hungary, *Isotope Laboratory, Biological Research Centre of the Hungarian Academy of Sciences, Szeged, Hungary and †Institute of Biochemistry, Nuclear Research Centre, Saclay, France

(Received 29 April 1981)

SUMMARY

The biological half-life of synthetic, radiochemically pure, biologically active $[^3]$H$8$-arginine-vasopressin ($[^3]$HAVP), the distribution of radioactivity among the organs and the in-vivo metabolism of the hormone were studied in the rat. The half-life calculated from the $[^3]$HAVP radioactivities isolated from the blood was found to be $1.74 \pm 0.22$ (s.d.) min in the fast phase, and $16.98 \pm 1.01$ min in the slow phase. The half-lives of total radioactivity were longer in both phases. The radioactivity accumulated to the greatest extents in the adenohypophysis and small intestine. The radioactive substance was accumulated more by the kidney than by the liver, but the hormone underwent inactivation more quickly in the liver.

INTRODUCTION

Vasopressin is an important hormone in mammals and has a considerable role in the regulation of a number of vital processes. To study its mechanism of action we felt it was necessary to know how it is cleared from the blood and we also wanted to collect data on its in-vivo metabolism. The low hormone level and its rapid degradation normally necessitate the use of a considerable multiple of the concentration of the physiological hormone. A concentration approaching that found under natural conditions has been attained with vasopressin labelled with a radioactive iodine isotope (Silver, Schwartz, Fong, Debons & Dahl, 1961; Klein & Roth, 1967; Baumann & Dingman, 1976; Shade & Share, 1976), but the biological activity of the derivative was only a fraction of that of the unlabelled hormone. On the other hand, in experiments with tritium-labelled vasopressin, although the amount of hormone employed was similarly higher than the physiological level, this compound had full biological activity (Silver et al. 1961; Sjöholm & Rydén, 1967; Willumsen & Bie, 1969; László, 1979).

Attempts to follow the processes of clearance and metabolism by bioassay are complicated (Schröder & Rott, 1959; Czaczkos & Kleeman, 1964; Fabian, Forsling, Jones & Pryor, 1969) and unreliable, while studies involving radioimmunoassay without a sufficiently specific antiserum have not given accurate results (Beardwell, 1971; Robertson, Mahr, Athar & Sinha, 1973; Skowsky, Rosenbloom & Fisher, 1974).

Hormone passing out of the circulation is rapidly metabolized by various organs (e.g. liver and kidney). The undecomposed vasopressin has been determined by bioassay, by radioimmunoassay and by the measurement of radioactivity (Walter & Simmons, 1977), but identification of the metabolites is almost impossible without the use of isotopically labelled compounds. The use of tritiated vasopressin provides a reasonable compromise between the dosage necessary for detection and the retention of biological activity.
Only limited data on tritiated 8-arginine-vasopressin ([3H]AVP) have been reported to date (Silver et al. 1961; Floreut, Terada, Yang, Nakagawa, Nakahara & Hechter, 1977) because of the methodological difficulties in its preparation and purification. In our earlier studies (László, 1979; László, Janáky, Beláspi & Morgat, 1981) we have investigated tritiated 8-lysine-vasopressin ([3H]LVP) and 1-deamino-8-arginine-vasopressin ([3H]DDAVP). It seemed to be necessary, therefore, to extend the experiments to AVP, which is the natural hormone in the rat.

This publication reports a study of the organ distribution and disappearance from the circulation of biologically active [3H]AVP.

**MATERIALS AND METHODS**

Male albino R-Amsterdam rats (Institute for the Breeding of Laboratory Animals, Gödöllő, Hungary) weighing 220–250 g, and maintained on a standard diet but with tap water available ad libitum, were used for the examinations.

**Radioactive material**

The [3H]AVP was prepared from synthetic 3,5-diiodotyrosine-2-8-arginine-vasopressin (Ferring AB, Malmö, Sweden) by catalytic tritiation (Morgat, 1979). The product was purified by thin-layer chromatography and high-performance liquid chromatography (HPLC). During the period of the experiments, the labelled hormone was stored in liquid air. 1-[3,5-3H]Tyrosine (The Radiochemical Centre, Amersham) with a specific activity of 244 mCi/mg (9·0 GBq/mg) was used as control.

**Measurement of biological activity**

The biological activity of the [3H]AVP was measured by the procedure of de Wied (1960) in female homozygous Brattleboro rats (Centraal Proefdierenbedrijf TNO, Zeist, The Netherlands) anaesthetized with alcohol. The antidiuretic effect of the [3H]AVP was compared with that of synthetic AVP (Organon, The Netherlands).

**High-performance liquid chromatography**

The high-performance liquid chromatograph consisted of a Model 6000 A pump (Waters GmbH, Vienna, Austria) and a universal liquid chromatograph injector (Model U6K), coupled to an LKB Uvicord III (Bromma, Sweden) fixed-wavelength u.v. monitor with an 8 µl flow-through cell and an LKB flat-bed recorder. Chromatography was performed using a reversed-phase Nucleosil 5C18 column (25·0 × 0·46 cm) packed with 5 µm octadeccysilica (Chrompack, Middelburg, The Netherlands). Methanol was used as supplied by Merck (Darmstadt, Germany). Water was glass-distilled and deionized. Elution was effected isocratically using 0·05 M-ammonium acetate buffer (pH 6·5)–methanol (60:40, v/v) (Lindeberg, 1980). The buffer solution was 0·05 mol/l in total acid and was freshly prepared by titration of aqueous acetic acid with ammonia solution to the desired pH. Aqueous solution was passed through an 0·45 µm membrane filter (Sartorius Membranfilter GmbH, Göttingen, Germany; catalogue no. 11405). Before use, the mobile phase was degassed for 5 min in an ultrasonic bath. Elution was performed for 15–20 min at room temperature and at a flow-rate of 1·2 ml/min. Column eluates were monitored for absorbance at 206 nm. Fractions of 1·2 ml were collected in counting vials, mixed with 10 ml scintillation mixture of toluene–Triton X-100 (2:1, v/v) containing 50 mg POPOP/l and 4 g PPO/l (in toluene), and counted with a Packard liquid scintillation spectrometer (Model 3255, Packard Instrument GmbH, Vienna, Austria). Quench correction was carried out with external standard ratios.

**Determination of biological half-life**

For investigation of the disappearance of the radioactivity from rat plasma, a polyethylene
cannula was inserted into the right carotid artery of ether-anaesthetized rats and diluted heparin was injected. The femoral vein was exposed and 15 μCi (555 kBq) [3H]AVP in a volume of 200 μl was administered. Blood samples were then taken through the carotid cannula after 20 and 40 s and 1, 2, 4, 8, 16, 32 and 60 min. The plasma was obtained by microcentrifugation, 50 μl was added to 10 ml Insta-Gel (Packard Instrument GmbH) and the radioactivity was determined.

To establish whether this radioactivity originated from the [3H]AVP itself or from one or another metabolite, the in-vivo clearance of the labelled hormone was also determined using another method (Witter, Scholten & Verhof, 1980). Rats were anaesthetized i.p. with 112.5 mg urethane in 0.9 ml 0.9% NaCl (w/v) solution per 100 g body weight. Using the method described above, 15 μCi [3H]AVP were administered. After various intervals (0-5, 1, 2, 4, 8, 16, 32 and 60 min) the cannula was rinsed out with two drops of blood, 200 μl blood were taken and added immediately to 2 ml ice-cold 6% (w/v) aqueous trichloroacetic acid (TCA) solution, and the solution was mixed well. After centrifugation (104 g, 15 min, 4°C), the precipitate was thoroughly washed with 2 × 2 ml 6% TCA solution, and the combined supernatant fractions were extracted with 3 × 10 ml ether to remove the TCA, and then lyophilized. The residues were dissolved in 200 μl water and filtered through a 0.45 μm Sartorius membrane filter. Analysis was performed by HPLC on 45–75 μl of the pure solution. Before chromatography, 10 μg (5 μl) standard AVP was added to every sample for determination of the retention time. Fractions (1.2 ml) were collected every minute and the radioactivity was measured. It was established from the blood taken at each point of time the percentage of radioactivity which originated from the intact [3H]AVP. The recoveries of [3H]AVP and [3H]tyrosine were checked following TCA extraction. A significant difference was not found between the two substances after extraction from four plasma samples each: the percentage recoveries of [3H]AVP and of [3H]tyrosine were 85.0 ± 5.25 (s.d.) and 84.3 ± 3.05 respectively.

For calculation of the biological half-life, the logarithms of the radioactivities measured after the different intervals were plotted against time. The regression line fitted to the points of the slow phase was extended to the ordinate and the values (disintegrations/min) corresponding to the experimental intervals on the extrapolated straight line were subtracted from the initial radioactivity values (disintegrations/min). The regression line fitted to the resulting points gave the fast phase. The half-life (t1/2) was calculated from the slope of the straight line relating the natural logarithms of the radioactivities to time (t1/2 = ln 2/slope).

Organ distribution of [3H]AVP

One hour after the administration of [3H]AVP the animals were decapitated, the organs rapidly removed and the blood contamination washed off with cold physiological saline solution. The smaller organs in their entirety, and 100 mg tissue from the larger organs, were incubated for 12 h at 56°C in 1 ml Soluene 350 (Packard Instrument). To decolourize the solution, 0.5 ml 30% H2O2 was added to the samples followed by 10 ml Dimilume-30 (Packard Instrument). After 3 days the radioactivity was measured with a Packard liquid scintillation spectrometer. The results were expressed as a percentage of the total radioactivity added measured in 100 mg wet weight of organ. In those organs in which the metabolism of vasopressin occurs (kidney, liver, small intestine), a study was made of the extent to which the accumulated radioactivity originated from the undecomposed [3H]AVP. The [3H]AVP (15 μCi) in 200 μl physiological saline solution was administered through the femoral vein to urethane-anaesthetized rats. At certain times (2, 5 or 60 min) one of the kidneys, one lobe of the liver, and a portion of small intestine were simultaneously removed from the animals and immediately placed in liquid air. Samples (200–400 mg) from these organs were homogenized (Ultra-Turax, Janke und Kunkel AG, Ika Werk, Staufen, Germany) with 3 ml 5% aqueous acetic acid at 0°C, the homogenizer
was rinsed with 1 ml 5% acetic acid, and the proteins were precipitated with 0.5 ml 30% TCA solution. After centrifugation (104 g, 15 min, 4°C) the supernatant fraction was removed, the precipitate washed with 2 × 1 ml 6% TCA solution, and the combined supernatant fractions were extracted with 4 × 10 ml ether to remove the TCA. After lyophilization, dissolution and filtration, the radioactivities of the tissue samples were analysed by HPLC by the method described above. Additionally, the organ distribution of [3H]tyrosine was determined in the manner employed for [3H]AVP.

The radioactivity of urine taken from the bladder at the end of the experiment was determined in a similar manner.

RESULTS

The chromatographic purity of the [3H]AVP was 97.2% as determined by HPLC. The retention time of [3H]AVP was 11.2 min, the same as for authentic AVP. The antidiuretic activity of the labelled hormone was compared with that of synthetic AVP, the biological activity of which was 411.3 ± 26.7 (S.E.M.) k.i.u./g. From ten determinations the biological activity of the [3H]AVP was found to be 397.2 ± 19.8 k.i.u./g. The specific activity of the purified [3H]AVP was determined by measurement of the biological activity of hormone of known radioactivity, i.e. 8.65 Ci/mmol (320 GBq/mmol). It may be stated that the [3H]AVP proved biologically active and radiochromatographically pure.

For investigation of the disappearance of the radioactivity from the rat plasma, the radioactivities measured at the individual points of time were plotted separately for every animal on semilogarithmic paper. Figure 1a shows the disappearance curve of radioactivity for the rat best approximating to the average, where the values represent the total radioactivity originating from both the [3H]AVP and the labelled metabolites together. Line 1 denotes the elimination (slow) phase, which is the regression line fitted to the radioactivities measured after 8, 16, 32 and 60 min (r ≥ 0.95). Line 2 shows the distribution (fast) phase, this being the regression line fitted to the points measured after 20 s, 40 s and 1, 2 and 4 min, corrected by subtraction on the basis of the slow phase (r ≥ 0.98). Using the data from ten animals the half-lives of the total radioactivity were found to be 2.49 ± 0.51 (S.D.) min (fast phase) and 27.9 ± 5.08 min (slow phase).

Figure 2 shows the variation in the distribution of the total radioactivity among the components with time. It may be stated that the [3H]AVP was initially metabolized extremely rapidly: apart from the vasopressin, two additional radioactive peaks appeared in the HPLC recordings after only 30 s. One eluted with the solvent front (this was proved by thin-layer chromatography to be [3H]tyrosine) and the size of the peak increased with time, while the other peak (retention time = 7.0 min) diminished. The quantity of [3H]AVP measured decreased with the passage of time.

The disappearance curve of intact [3H]AVP is shown in Fig. 1b. From these data the biological half-lives of vasopressin were 1.74 ± 0.22 (S.D.) min (n = 3) in the fast phase (line 2) and 16.98 ± 1.01 min in the slow phase (line 1). These half-lives were shorter than the values calculated from the total radioactivity.

The average maximum initial plasma concentration of [3H]AVP was 93.18 ± 7.30 (S.D.) nCi (107 pmol)/50 µl plasma calculated as the dose administered (15 µCi) divided by the plasma volume (6–8 ml). The latter volume was obtained according to the method of Ginsburg & Heller (1953), who found the rat plasma volume to be 33.7 ml/kg body weight. However, the value obtained by extrapolation of the disappearance curve of [3H]AVP to the zero point was 31.22 ± 7.49 nCi (3.6 pmol)/50 µl plasma. Fast diffusion can explain this large difference between the two values.

The data relating to organ distribution of radioactivity are listed in Table 1. After [3H]AVP administration the radioactivity accumulated to the greatest extent in the adenohypophysis, small intestine, neurohypophysis and kidney. Only minimal radio-
Fig. 1. Disappearance of (a) radioactivity and (b) intact $[^3]$H-arginine-vasopressin ($[^3]$HAVP) from the plasma of a rat after intravenous administration of $[^3]$HAVP. The half-life of the total radioactivity for the fast phase (line 2) was 2.49 ± 0.51 (s.d.) min and for the slow phase (line 1) 27.9 ± 5.08 min ($n=10$). From the values in three animals, the average half-life of the intact $[^3]$HAVP was found to be 1.74 ± 0.22 min in the fast phase, and 16.98 ± 1.01 min in the slow phase.

Table 1. Mean (± S.E.M.) distribution of radioactivity (% total activity administered/100 mg organ weight) in various organs of rats

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>0.129 ± 0.034</td>
<td>0.357 ± 0.023*</td>
</tr>
<tr>
<td>Liver</td>
<td>0.090 ± 0.013</td>
<td>0.105 ± 0.011</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.031 ± 0.007</td>
<td>0.044 ± 0.003</td>
</tr>
<tr>
<td>Small intestine</td>
<td>0.195 ± 0.049</td>
<td>0.108 ± 0.009</td>
</tr>
<tr>
<td>Cerebral cortex</td>
<td>0.042 ± 0.009</td>
<td>0.045 ± 0.002</td>
</tr>
<tr>
<td>Neurohypophysis</td>
<td>0.140 ± 0.026</td>
<td>0.297 ± 0.015*</td>
</tr>
<tr>
<td>Adenohypophysis</td>
<td>0.227 ± 0.051</td>
<td>0.088 ± 0.006*</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>0.043 ± 0.008</td>
<td>0.031 ± 0.003</td>
</tr>
</tbody>
</table>

* $P<0.01$ compared with control rats (Student's $t$-test).
Fig. 2. Distribution of radioactivity in rat blood after intravenous administration of \[^{3}\text{H}8\text{-arginine-vasopressin}\] (\[^{3}\text{H}AVP\]) (line 1), labelled intermediate metabolite (line 2) and \[^{3}\text{H}tyrosine\] (line 3) after high-performance liquid chromatography of blood extracts (\(n = 3\)). Values are means ± s.d.

Table 2. Variation of distribution of radioactivity in time as percentage of total radioactivity (three rats). Values are means ± S.E.M.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>[^{3}\text{H}\text{Arginine-vasopressin}]</th>
<th>[^{3}\text{H}\text{Tyrosine}]</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>5.4 ± 1.05</td>
<td>93.4 ± 1.30</td>
</tr>
<tr>
<td>5</td>
<td>2.3 ± 0.26</td>
<td>96.4 ± 0.59</td>
</tr>
<tr>
<td>60</td>
<td>&lt;0.1</td>
<td>&gt;99.0</td>
</tr>
</tbody>
</table>

activity was measured in the cerebral cortex, hypothalamus and muscle. After the administration of \[^{3}\text{H}tyrosine\] the greatest accumulation was found in the kidney and neurohypophysis, with much lower levels in the small intestine and adenohypophysis. The other data were almost the same as those measured in the case of \[^{3}\text{H}AVP\].

The HPLC examinations showed that the radioactivity originated not from the unchanged \[^{3}\text{H}AVP\], but from the \[^{3}\text{H}tyrosine\]. The radioactivity data obtained after 2 and 5 min indicated that the vasopressin underwent inactivation most rapidly in the small intestine, followed in turn by the liver and the kidney. Of the radioactive substances, only \[^{3}\text{H}AVP\] and \[^{3}\text{H}tyrosine\] were found (Table 2). In the urine taken 1 h after the \[^{3}\text{H}AVP\] administration, a considerable quantity of radioactivity was found; 11% of this originated from undecomposed \[^{3}\text{H}AVP\], 16% from the intermediate metabolite, and the remainder from \[^{3}\text{H}tyrosine\] eluting together with the solvent front.

**DISCUSSION**

In contrast with the bioassay and radioimmunoassay employed in the study of vasopressin excretion, experiments involving vasopressin labelled with a radioactive isotope have yielded much more information. Although the use of \(^{125}\text{I}\) and \(^{131}\text{I}\)-labelled vasopressin
allowed investigations to be carried out under concentration levels approximating to the physiological level of hormones (Silver et al. 1961; Klein & Roth, 1967; Baumann & Dingman, 1976; Shade & Share, 1976), the labelling with iodine isotope meant a loss in biological activity (Fong, Silver, Christman & Schwartz, 1960). Only LVP (László, 1979; Sjöholm & Yman, 1967; Willumsen & Bie, 1969) and synthetic dDAVP (László et al. 1981) have been labelled with tritium and examined. Although a hormone concentration higher than the natural one was used in these experiments, the biological activities were complete. Flouret et al. (1977) prepared [3H]AVP for receptor binding. Silver et al. (1961) dealt with [3H]AVP; they found the half-life of the hormone to be 2·3–5·5 min in man and 4–5 min in dogs. By measurement of the anti-diuretic activity, the biological half-life of AVP was found by Aziz (1969) to be 3·3–4·2 min and by Smith & Thorn (1965) to be 1·0 min, while by measurement of the pressor activity Gazis & Sawyer (1978) obtained a value of 2·6 min in rat. In experiments with Pitressin (a mixture of AVP and LVP) Lauson (1974) determined a half-life of 0·85–4·2 min.

The results of our investigations agree with the above values, but it should be noted that the authors cited above carried out analyses only on the fast phase and not on the slow phase. This is of particular importance because when the half-life is determined in an identical manner, a significant difference between the individual analogues (AVP, LVP and DDAVP) is observed only in the slow phase (T. Janáky, J. Gyöngyösi, F. A. László & M. Sas, unpublished observations).

Decrease of the intact vasopressin level in the blood is extremely rapid after intravenous administration of the hormone. It is likely that the disappearance during the first 2 min is due to reversible outward diffusion to the tissues as well as irreversible inactivation (Sjöholm & Rydén, 1967). This concept is supported by our finding that the vasopressin level extrapolated to zero time is approximately one-third of the expected value, while the inactivation is also fast: 2 min after injection of [3H]AVP only 58% of the plasma radioactivity is in the form of undecomposed [3H]AVP. The discrepancy between the biological half-lives calculated from the disappearance of the total radioactivity and the decrease of the intact AVP level may be due to the different metabolic clearances of the compounds ([3H]AVP and metabolites).

When considering the metabolism of hormones, theoretically it is necessary to take into account excretion from the circulation, binding to plasma proteins, outflow into the tissues, accumulation there, and enzymatic inactivation. In in-vitro experiments the plasma of control rats decomposed the vasopressin only slowly, and thus it is probable that the plasma does not participate directly in the metabolism of the hormone (Fabian, Forsling, Jones & Lee, 1969). Hormone metabolism may also be influenced by the blood circulation conditions. The vasopressin dose administered was larger than the pharmacological dose; it caused the blood pressure of the rats to increase by 25–50 mmHg, with a return to the initial value after 50–100 s. It is probable that the cardiovascular effect of the large dose of vasopressin changes the diffusion in the fast phase, but the half-life determined on the basis of the slow phase is characteristic of the substance. There is no correlation between the blood flow through the various organs and the distribution of radioactivity. For instance, the blood flow of the rat adenohypophysis is 0·76 ± 0·06 (s.e.m.) μl/min per mg tissue, while that of the neurohypophysis is 5·05 ± 0·73 μl/min per mg tissue (David, Csernay, László & Kovács, 1965); at the same time the radioactivity is accumulated to the greatest extent in the adenohypophysis.

From our examinations of the organ distribution of [3H]AVP and the accumulation of the radioactivity in the tissues, it may be stated that high activity was observed in those organs (kidney and liver) in which the vasopressin is quickly metabolized. However, attention must be drawn to the considerable accumulation in the small intestine, an amount exceeding that in the kidney and the liver. The experiments of Larson (1938) demonstrated that an extract of small intestinal mucosa destroys the pressor activity of vasopressin.
The data from high-performance liquid chromatograms prepared after 2 and 5 min indicated that inactivation of the hormone was fastest in the small intestine, and the most radioactivity was accumulated here after 1 h. This observation appears to contradict the results of Lauson, Bocanegra & Beuzeville (1965), who found that one-third of the circulating vasopressin is removed from the circulation by the liver and two-thirds of it by the kidney. Our data suggest that the metabolic activity is more marked in the kidney than in the liver and thus, in agreement with the observations of other authors, the majority of the hormone is removed from the circulation by the kidney (Little, Klevay, Radford & McGandy, 1966; Rabkin, Ghazeleh, Share, Crofton & Unterhalter, 1980). The rate of inactivation, however, is more pronounced in the liver (Dicker & Greenbaum, 1956); this is supported by our radiochromatograms taken after 2 and 5 min: after 2 min the \(^{3}H\)AVP accounts for 5-4% of the radioactivity in the kidney but only 2-7% in the liver. The phenomenon may be explained by the different enzymatic activities, or by the fact that there are specific vasopressin receptors in the kidney, whereas only non-specific membrane binding is to be found in the liver (Rabkin et al. 1980).

It is noteworthy that the highest radioactivity accumulation is to be observed in the adenohypophysis and neurohypophysis. A marked accumulation of \(^{3}H\)LVP was likewise found in these organs earlier (László, 1979). Our present data are in accordance with the observations by Willumsen & Bie (1969), who measured considerable radioactivity in both organs, 2 and 12 min after the intravenous administration of \(^{3}H\)LVP. Pliška, Thorn & Vilhardt (1971) reported that the neurohypophysis also contains vasopressin-inactivating aminopeptidase and trypsin-like enzymes.

REFERENCES


Metabolism and organ distribution of \(^3\text{H}\)AVP in rats


