BIOLOGICAL HALF-LIFE AND ORGAN DISTRIBUTION OF 
$[^3\text{H}]1$-DEAMINO-8-D-ARGININE-VASOPRESSIN IN THE RAT

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SUMMARY
The biological half-life of synthetic, radiochemically pure, biologically active $[^3\text{H}]1$-deamino-8-d-arginine-vasopressin (dDAVP) in rats was found to be 5:33 ± 0:28 (s.e.m.) min in the initial, transitional, fast phase and 56:28 ± 3:27 min in the second, slow phase. The substance accumulated to the greatest extent in the kidney and small intestine and only slightly in the adenohypophysis. The results have suggested that the extended biological half-life may play a role in the more marked and longer antidiuretic effect of dDAVP. The explanation of the poor accumulation in the adenohypophysis may be that dDAVP does not possess an effect similar to that of corticotrophin releasing factor.

INTRODUCTION
The vasopressin derivative, 1-deamino-8-d-arginine-vasopressin (dDAVP), is widely and effectively used in clinical practice for the treatment of diabetes insipidus (Zaoral, Kolc & Sorm, 1967; Andersson & Arner, 1971, 1972; Aronson, Andersson, Bergstrand & Mulder, 1973; Nash, 1973; Kauli & Laron, 1974; Némethova & Lichardus, 1974; Ward & Fraser, 1974; Lebacq & David, 1975). It is known that dDAVP has a slight pressor effect, whereas its antidiuretic activity is several times that of the natural hormone and the duration of its action is considerably longer (Walter, Rudinger & Schwartz, 1967; Sawyer, Acosta, Baláspiri, Judd & Manning, 1974; Robinson, 1976; Czakó, Julesz & László, 1978; Seif, Zenser, Ciarochi, Davis & Robinson, 1978). The difference between the biological effects raises the question of whether the variation in the vasopressin molecule is accompanied by a change in the metabolism of the hormone and this has been studied using biologically active, tritiated dDAVP. The biological half-life and organ distribution of the labelled hormone were examined.

MATERIALS AND METHODS
Male albino R-Amsterdam rats (Institute for the Breeding of Laboratory Animals, Gödöllő, Hungary) weighing 180–200 g, kept on a standard diet but with tap water available ad libitum, were used for the examinations.

Radioactive material
Tritiated dDAVP ($[^3\text{H}]d\text{DAVP}$) was prepared after the iodination of synthetic dDAVP (synthesized by the method of Baláspiri, Tóth & Kovács, 1979) by catalytic substitution of the iodine atoms with tritium. The tritium was incorporated into the molecule in place of the iodine atoms on the aromatic ring of the tyrosine, the second amino acid. The compound...
proved to have a specific activity of 15 Ci/mmol. Details of the preparation and purification of [3H]dDAVP are to be found in Morgat (1979).

Check on radiochemical purity
Before the experiment and following the completion of the examinations, the homogeneity of the radioactive hormone was investigated. For this purpose, 20 µg non-radioactive synthetic dDAVP and about 200 000 disintegrations [3H]dDAVP/min were taken at the starting-point of a silica-gel G thin-layer chromatogram, and subjected to chromatography in a mixture of n-butanol, water and glacial acetic acid (8 : 5 : 4, by vol.). The chromatogram was developed with ninhydrin reagent (E. Merck, Darmstadt, Germany) and its radioactivity then examined with a Packard chromatogram scanner. The radiochemical purity of the [3H]dDAVP and the coincidence of the radio and biological activities were also checked using the following method. Tritiated dDAVP was chromatographed on a 1·5 × 25 cm Sephadex G-15 column with 0·2 N-acetic acid solution, at a flow-rate of 8 ml/min. The individual fractions had a volume of 1 ml. The radio and biological activities of each tube were determined separately.

Measurement of biological activity
The biological activity was measured by the procedure of de Wied (1960) in alcohol-anaesthetized female homozygous Brattleboro rats (Centraal Proefdierenbedrijf TNO, Zeist, The Netherlands) weighing 160–180 g. The antidiuretic effect of the [3H]dDAVP was compared with that of synthetic dDAVP, the biological activity of which was 955 ± 38·5 (s.e.m.) i.u./mg. Ten determinations in each case showed the biological activity of the [3H]dDAVP to be 928 ± 47·3 i.u./mg before the experiment and 907 ± 45·3 i.u./mg after the investigations. During the examinations the labelled material was stored in liquid air and remained usable for about 4 months.

Determination of biological half-life
The biological half-life was determined with a micro-method. 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 [3H]dDAVP in a volume of 0·2 ml was administered. Following this, blood samples were taken through the carotid cannula after 20, 40, 60, 120, 240 and 480 s and 16, 30 and 60 min; these samples, in 0·1 ml capillary tubes, were centrifuged to separate the plasma from the formal elements. Plasma (0·05 ml samples) was blown into 10 ml toluene solution containing Triton X-100 : PPO + POPOP (ratio, 1 : 2) and the radioactivity was measured with a Packard Tricarb liquid scintillation spectrometer. The activities of the individual samples were given in disintegrations/min. The biological half-life was calculated using the method of Lauson (1974).

Measurement of radioactivity of organ extracts
At the end of the examination the rats were decapitated, the organs rapidly removed and the blood contamination washed off the larger organs in cold physiological salt solution. The organs were weighed, homogenized, transferred to 1–2·5 ml Soluene 350 (Packard Instrument, Vienna, Austria; the volume depending on the amount of tissue) and incubated for 12 h at 56 °C. To decolourize the solution, 0·5 ml H2O2 was added per ml dissolved tissue extract, followed by 10 ml Triton X-100 liquid scintillation fluid. After 3 days the radioactivity was determined with a Packard liquid scintillation spectrometer. The results were expressed as a percentage of the total radioactivity added, calculated on 100 mg wet weight of organ. Before the serial examinations and for purposes of information only the radioactivities of the organ extracts were determined 15, 30, 60 and 120 min after administration of [3H]dDAVP to two rats in each case; it was decided that determination after 1 h was the most appropriate, for at this time the radioactivity in the organs studied was at its highest value.
RESULTS

The $[^3\text{H}]dDAVP$ was pure from a radiochromatographic aspect: after thin-layer chromatography one peak was visible, coinciding with the spot of authentic dDAVP developed with ninhydrin. In both cases the relative front value proved to be 0.50. The same result was obtained when the thin-layer chromatographic procedure was repeated at an interval of about 4 months after completion of the experimental period.

The data obtained by chromatography on the Sephadex G-15 column are shown in Fig. 1. It may be observed that the radioactivity of the tubes was accumulated in one peak (between tubes 31 and 43). The biological activity could be demonstrated in the same peak: the radioactivity was parallel with the biological activity. These results indicated that the $[^3\text{H}]dDAVP$ was biologically active and homogeneous from a radiochemical aspect.

The biological half-life was determined by plotting the radioactivities of the individual plasma samples on semilogarithmic paper. Joining the points yielded two straight lines, from which the 100% value was obtained by extrapolation to zero time. The biological half-life was determined separately in each animal. Figure 2 shows the plot for the rat best

\[ \text{Radioactivity (disintegrations/min per ml)} \]

\[ \text{Biological activity (i.u./l)} \]

Number of tubes

10 20 30 40 50 60 70

\[ 2 \times 10^5 \]

\[ 1 \times 10^5 \]

\[ -15 \]

\[ -10 \]

\[ -5 \]

\[ 100 \]

\[ 80 \]

\[ 60 \]

\[ 40 \]

\[ 30 \]

\[ 20 \]

\[ 10 \]

0 4 8 16 32 60

\[ \% \text{ of zero-time radioactivity} \]

\[ \text{Time after injection (min)} \]

10 20 30 40 50 60

\[ 100 \]

\[ 80 \]

\[ 60 \]

\[ 40 \]

\[ 20 \]

\[ 10 \]

Fig. 1. Biological activity (○) and radioactivity (●) of $[^3\text{H}]$-deamino-8-D-arginine-vasopressin after Sephadex G-15 chromatography.

Fig. 2. Biological half-life of $[^3\text{H}]$-deamino-8-D-arginine-vasopressin in one rat. From the values obtained in ten animals, the average half-life for the first phase was 53.33 ± 0.28 (s.e.m.) min and for the second 56.28 ± 3.27 min.
approximating to the average. It may be stated that, in accordance with the two components of the curve, the transitional, fast phase of decrease in the radioactivity can be seen first, followed by the second, protracted, slow phase. From the values obtained on ten animals, the average half-life for the fast phase was found to be $5.33 \pm 0.28$ min and that for the slow phase $56.28 \pm 3.27$ min.

The data relating to organ distribution are listed in Table 1, which reveals that most radioactivity could be detected in the kidney, followed by the small intestine and then the liver, neurohypophysis and adenohypophysis. Only very low radioactivity was observed in muscle, hypothalamus and cerebral cortex.

Table 1. Mean (±s.e.m.) distribution of $[^3\text{H}]d\text{DAVP}$ radioactivity (total activity %/100 mg organ weight) in various organs of ten rats

<table>
<thead>
<tr>
<th>Organ</th>
<th>Radioactivity</th>
<th>Organ</th>
<th>Radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>0.83 ± 0.05</td>
<td>Neurohypophysis</td>
<td>0.20 ± 0.03</td>
</tr>
<tr>
<td>Liver</td>
<td>0.20 ± 0.01</td>
<td>Adenohypophysis</td>
<td>0.14 ± 0.01</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.07 ± 0.01</td>
<td>Hypothalamus</td>
<td>0.06 ± 0.01</td>
</tr>
<tr>
<td>Small intestine</td>
<td>0.57 ± 0.05</td>
<td>Cerebral cortex</td>
<td>0.08 ± 0.01</td>
</tr>
</tbody>
</table>

To examine the extent to which the radioactivity originated from the hormone itself, the biological activities of extracts of liver, small intestine and kidney tissues were determined and the procedure of Pliska, Barth & Thorn (1971) was followed to identify the dDAVP by thin-layer chromatography. It was found that 1 h after the injection of $[^3\text{H}]d\text{DAVP}$ one-third of the radioactivity accumulated in the liver and in the small intestine and one-tenth of that in the kidney originated from the non-metabolized hormone; this was confirmed by the biological activities measured.

**DISCUSSION**

By determination of the pressor activities, Gazis & Sawyer (1978) found the biological half-lives of arginine-vasopressin and lysine-vasopressin (LVP) in the rat to be 2.9 and 3.2 min respectively; this latter value corresponds to the one reported earlier (László, Nagy, Gáspár, Kéri & Teplán, 1980). The biological half-life of dDAVP has proved to be longer; in our examinations we found results similar to those of Edwards, Kitaú, Chard & Besser (1973) who measured a time of 7.8 min in the fast phase and 75.5 min in the slow phase.

The larger and more prolonged antidiuretic effect of dDAVP can be explained by the longer biological half-life. However, the metabolisms of the hormones do not depend only on their biological half-lives; among other factors it is necessary to take into account excretion, binding to plasma proteins, outflow into the tissues, accumulation, and the appearance of degradation products. We have investigated the organ distribution of $[^3\text{H}]d\text{DAVP}$ and the accumulation of the radioactivity in the tissues and it was found that, in comparison with $[^3\text{H}]\text{LVP}$ (László, 1979), appreciably more $[^3\text{H}]d\text{DAVP}$ was accumulated in the kidney and small intestine, but much less in the neurohypophysis and adenohypophysis.

It was earlier considered noteworthy (László et al. 1980) that a large amount of $[^3\text{H}]\text{LVP}$ accumulates in the adenohypophysis. Our data are in accordance with the observation of Willumsen & Bie (1969) who measured considerable radioactivity in both the adenohypophysis and the neurohypophysis 2 and 12 min after intravenous administration of the labelled hormone. This phenomenon tends to refocus attention on the conception that vasopressin, as one of the corticotrophic releasing factors (CRF), may play a role in the
enhancement of secretion of adrenocorticotropic hormone (ACTH) (McCann, Antunes-Rodrigues, Nallar & Valtin, 1966; Yates, Russel, Dallman, Hedge, McCann & Dharwiwal, 1971; Gillies, Van Wimersma-Greidanus & Lowry, 1978; Chateau, Marchetti, Buret & Boulange, 1979). This hypothesis is supported by our present observation that [3H]dDAVP, which does not exhibit a CRF-like effect (F. A. László, T. Janáky, G. Kártész & G. Makara; unpublished observations), accumulates to a lower extent than [3H]LVP in the adenohypophysis. It may be assumed that the adenohypophysis contains natural vasopressin receptors which are indifferent with regard to dDAVP. Direct adenohypophysial action of vasopressin is supported by those experiments in which the enhancing effect of the hormone on ACTH secretion was observed under in-vitro conditions (Portanova & Sayers, 1973; Pearlmutter, Rapino & Safran, 1974; Fehm, Voigt & Lang, 1975; Buckingham & Hodges, 1977; Gillies & Lowry, 1978).

REFERENCES


