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Plasma Half-Lives of Vasopressin and Oxytocin Analogs After iv Injection in Rats (40269)

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Although many workers have measured the plasma half-lives of oxytocin and vasopressin in rats (1), there have been few studies on the half-lives of structural analogs of vasopressin and oxytocin. This is largely due to the lack of suitable radioimmunoassay methods and the difficulties inherent in measuring peptides in plasma by bioassays. Studies on the rates of elimination from plasma of analogs of the neurohypophysial hormones could provide information on the manner in which the endogenous hormones are metabolized. I have, therefore, applied a new method for estimating plasma half-lives from vasopressor responses (2) to a series of analogs of vasopressin and oxytocin in an effort to determine which structural changes can delay elimination from the plasma. From such information one may speculate on the types of enzymatic inactivation that are important for the elimination of arginine-vasopressin and oxytocin and whether these are different for these two hormones.

Materials and methods. To screen for possible long-acting analogs, I first looked through several years' records of vasopressor assays done on vasopressin and oxytocin analogs in Dr. W. H. Sawyer's laboratory. If I could mask identification of the injections and consistently distinguish the responses to the test analog as longer than the responses to the standard, I considered the analog as a candidate for half-life studies. The analogs which could be so distinguished were [8-ornithine]-oxytocin, [8-arginine]-vasotocin, and deaminated analogs of vasopressin. Guided largely by this survey, I decided to concentrate on measuring the half-lives of analogs modified in the one position, the eight position, and the one and six positions.

Female Sherman rats (from Camm Research) were used throughout. They were prepared as for vasopressor assay, and the pressor responses to a series of intravenous injections of each analog were analyzed to obtain a plasma half-life by the curve-fit method, as previously described (2). The series of injections was 1, 2, 4, 8, and 32 pressor mU of peptide, given in ascending order of dose, in all instances. A new injection was given only after blood pressure had returned to normal for 10–15 min. Each rat was used to study two peptides. In the instances in which two peptides were found to have significantly different mean half-lives as determined from separate groups of rats, the longer-acting peptide was also longer-acting when the two peptides were compared in an individual rat.

Peptides used for half-life determinations were arginine-vasopressin, lysine-vasopressin, arginine-vasotocin, oxytocin, "1-hydroxy-arginine-vasopressin" ([1-L-2-hydroxy-3-mercaptopropionic acid]-arginine-vasopressin), "1-hydroxy-oxytocin", [8-ornithine]-oxytocin, [8-glutamine]-oxytocin, [8-proline]-oxytocin, "deamino-dicarba-arginine-vasopressin" ([1,6-aminosuberic acid]-arginine-vasopressin), and deamino-dicarba-arginine-vasotocin. Some of the data on half-lives of arginine-vasopressin, arginine-vasotocin, and deamino-arginine-vasopressin have been reported in a previous paper (2). I also investigated, but rejected for detailed half-life studies, [8-phenylalanine]-oxytocin and [8-isoleucine]-oxytocin, because their half-lives did not seem to be longer than that of oxytocin. Deamino-dicarba-oxytocin, given to five rats, was not included because it had such low pressor activity that only the lower part of the log-dose response curve could be obtained if reasonable injection volumes were used, and the half-lives thus obtained were not as accurate as they would have been if the entire log-dose response curve had been used.

Synthetic arginine-vasopressin (3), arginine-vasotocin (3), "1-hydroxy-arginine-vasopressin" (4), "1-hydroxy-oxytocin" (5), [8-glutamine]-oxytocin (6), [8-phenylalanine]-

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oxytocin (7), [8-proline]-oxytocin, [8-isoleucine]-oxytocin and lysine-vasopressin (M. Manning, unpublished) were all generously supplied by Dr. M. Manning of the Medical College of Ohio. Deamino-oxytocin was a gift from Dr. R. Walter of the University of Illinois Medical Center, and [8-ornithine]-oxytocin (8) was donated by Dr. B. Berde of Sandoz Ltd., Basel. Deamino-dicarba-arginine-vasopressin, deamino-dicarba-arginine-vasotocin, and deamino-dicarba-oxytocin were purchased from Peninsula Laboratories.

Results. Table I shows the mean half-life obtained for each analog. Of the naturally occurring peptides tested, lysine-vasopressin, arginine-vasopressin, and oxytocin have similar half-lives. However, arginine-vasotocin had a slightly but significantly ($P < 0.005$) longer half-life than arginine-vasopressin. When arginine-vasotocin was compared to arginine-vasopressin in individual rats, this difference was also consistently observed. Deamination lengthens the half-life of arginine-vasopressin considerably, that of vasotocin to a lesser degree, and that of oxytocin not at all.

When two carbons are substituted for the disulfide bond between residues 1 and 6, the half-lives of the resulting analogs, deamino-dicarba-vasopressin and deamino-dicarba-vasotocin, were not increased to a statistically significant extent over their respective deaminated analogs containing the disulfide group.

Substituting a hydroxyl group for the amino group in the first residue lengthened the half-life of arginine-vasopressin but had little or no effect on the half-life of oxytocin.

Some analogs of oxytocin with substitutions in the 8-position (arginine, ornithine, glutamine, or proline) have prolonged half-lives. To confine this study to a manageable number of analogs, we only did complete half-life studies on the 8-substituted oxytocin analogs with the longest half-lives. We obtained preliminary half-lives in a few rats on two other 8-substituted analogs, 8-phenylalanine and 8-isoleucine oxytocin. These seemed to have half-lives comparable to that of oxytocin.

Discussion. Deamination prolongs the half-life of arginine-vasopressin and that of arginine-vasotocin to a lesser degree, suggesting that inactivation of these peptides *in vivo* depends largely on aminopeptidase activity. Infusion studies (9) have also shown that aminopeptidase activity is important for vasopressin destruction. 95 ± 15% of infused deamino-arginine-vasopressin is excreted unmetabolized in the urine. Deamination of oxytocin does not prolong half-life. Nor does replacement of the amino group of residue 1 by a hydroxy group appear to protect oxyto-

TABLE I. Half-lives (in minutes) of analogs of vasopressin and oxytocin

Analog	Half-life	Number of rats
Lysine-vasopressin	3.2 ± 0.5 (SEM)	6
Arginine-vasopressin	2.9 ± 0.2	33
Arginine-vasotocin[a]	3.8 ± 0.2	30
Oxytocin	3.4 ± 0.4	14
Deamino-arginine vasopressin[a, d]	9.0 ± 1.2	11
Deamino-arginine-vasotocin[a]	5.5 ± 0.9	5
Deamino-oxytocin	3.3 ± 0.5	7
Deamino-dicarba-arginine-vasopressin[a, c]	11.4 ± 1.7	5
Deamino-dicarba-arginine-vasotocin[a]	5.9 ± 1.0	5
1-hydroxy-arginine-vasopressin[a, b]	6.7 ± 0.6	8
1-hydroxy-oxytocin	4.1 ± 0.4	14
[8-ornithine]-oxytocin[a, e]	5.5 ± 0.8	7
[8-glutamine]-oxytocin[a, e]	4.9 ± 0.4	7
[8-proline]-oxytocin[a, e]	4.9 ± 0.4	6

[a] Significantly different from arginine-vasopressin $P < 0.01$.
[b] Significantly different from 1-hydroxy-oxytocin $P < 0.02$.
[c] Significantly different from deamino-dicarba-arginine-vasotocin $P < 0.01$.
[d] Significantly different from deamino-arginine-vasotocin $P < 0.01$.
[e] Significantly different from oxytocin $P < 0.05$.

cin noticeably, although it prolongs the half-life of arginine-vasopressin. If one assumes that deamination or hydroxylation both interfere with inactivation by aminopeptidases, it would appear that aminopeptidase activity may not be necessary for rapid destruction of oxytocin analog. These results based on vasopressor responses appear to agree with previous observations on the duration of antidiuresis in diabetes insipidus rats in response to some of these analogs. Sawyer, Acosta, and Manning (10) found that deamination markedly prolonged the antidiuretic action of arginine-vasopressin and that of arginine vasotocin to a lesser extent. Deamination of oxytocin had no demonstrable effect on the duration of its antidiuretic action.

Unfortunately one cannot say much about dicarba substitution. In deamino-arginine-vasopressin and deamino-vasotocin it has no significant effect on half-life. But we do not know if dicarba substitution alone would prolong half-life, because I used deaminated dicarba analogs and their half-lives were presumably already prolonged by deamination.

The nature of the 8-amino acid appears to influence the metabolism of oxytocin analogs. Thus, one of the pathways for oxytocin metabolism appears sensitive to changes in the 8-position. Koida, Glass, Schwartz, and Walter (11) have partly purified an enzyme from kidney homogenates that inactivates oxytocin. Its ability to inactivate oxytocin analogs depends on the nature of the amino acid in the 8-position. Thus, this enzyme or similar enzymes may be important in the *in vivo* destruction of oxytocin.

Metabolic destruction is not the only determinant of plasma half-life. Differing rates of diffusion from the vascular compartment, different volumes of distribution, and binding to tissue or plasma proteins could also influence half-life. However, metabolic destruction is a major determinant of the plasma half-life of injected vasopressin and oxytocin (1), and resistance to enzymatic degradation is a likely explanation for the longer plasma half-lives of 1-substituted vasopressins and 8-substituted oxytocins.

Although it is not possible to reach conclusions about the importance of metabolism solely from curve-fit half-life measurements, studies such as the present one are a useful supplement to studies of vasopressin distribution and elimination, such as those reviewed by Lauson (1) and studies of degradation of oxytocin, vasopressin, and their analogs by tissue homogenates, such as those recently reviewed by Walter and Simmons (12).

Summary. The curve-fit method for estimating plasma half-lives from vasopressor responses in rats (2) was used to estimate the plasma half-lives of vasopressin and oxytocin analogs substituted in the 1, 1 and 6, and 8 positions. Deamino-vasopressin and 1-hydroxy-oxytocin did not seem to be long-acting. Deamino-dicarba analogs of arginine-vasopressin and arginine-vasotocin were long-acting to the same degree, respectively, as deamino-vasopressin and deamino-vasotocin. Three 8-substituted analogs of oxytocin, [8-ornithine]-oxytocin, [8-glutamine]-oxytocin, and [8-proline]-oxytocin, were also long-acting.

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