Responses of Rat Kidney and Toad Bladder to the Pentapeptide Ring Structures of Neurohypophysial Hormones[1] (37448)

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Neurohypophysial hormones, oxytocin and vasopressin, and their structural analogs have natriuretic and antidiuretic effects in the mammalian kidney (1–6). These peptides also have a marked natriferic (on sodium transport) and hydro-osmotic (on water permeability) effects on the amphibian urinary bladder (7–9). There have been numerous studies of structure–activity relationships of these peptides attempting to elucidate the molecular requirements for such specific activities. Although a generalized conclusion cannot be drawn an apparently strong correlation emerges between activities affecting sodium transport and the presence of an unaltered oxytocin pentapeptide ring structure in the molecule (10, 11). Most previous studies were done on octapeptide analogs. A number of cyclic pentapeptides corresponding to the ring structure of oxytocin or vasopressin have recently been synthesized (12–14) and it is now possible to assess directly the relative importance of the ring structures of oxytocin and vasopressin to their biological activities. In this paper, we report our findings on the water and sodium transport activities of six pentapeptide ring structures of the neurohypophysial hormones in the rat kidney and the toad urinary bladder.

Materials and Methods. For determination of antidiuretic and natriuretic activities in rats. Male Sherman albino rats weighing between 250 and 350 g were used. They were anesthetized initially with ethyl-(1-methylpropyl)-thiobarbiturate (Inactin) 7 mg/100 g ip. Anesthesia was subsequently maintained by 2.0% ethanol given orally in the hydration mixture. Atropine 0.2 mg/100 g sc was also administered. Tracheostomy was performed and one jugular vein was cannulated for iv injections. The urinary bladder was cannulated through an abdominal incision. Methods for induction of maintained water diuresis and for antidiuretic assays were described by Sawyer (15). The oral waterload was kept constant at 8% of the body weight. The hydrating solution was a mixture of 0.3% NaCl, 0.5% dextrose and 2.0% ethanol. The USP Posterior Pituitary Reference Standard was used as the standard in the antidiruetic assays.

The natriuretic potencies of the cyclic pentapeptides at a fixed dose of 0.7 μg/100 g iv were compared in rats under water diuresis. Only one peptide was tested in each animal. Urine samples were collected at 3 min intervals. Urinary sodium was determined by flame photometry.

For determination of hydro-osmotic and natriferic activities in the toad urinary bladder. Hydro-osmotic activity was estimated by Bentley's method (8) as modified by Sawyer (16). Toad (*Bufo marinus*) bladders were used. Half-bladders were tied onto glass tubes, forming sacs with the serosal surface outwards. Each sac was filled with 5 ml of distilled water and suspended in a 25 ml bath of Ringer's solution equilibrated with room air at room temperature. Water movement was determined by weighing each sac every

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15 min until a relatively stable rate was attained. The test peptide was then added to the serosal medium for a 30 min test period. The rate of water loss (rate of loss in weight of the bladder sac) between 15 and 30 min after the addition of the peptide was taken as the response.

Natriferic activity was measured by the "short-circuit" current method as described by Ussing and Zerahn (17). A half-bladder was mounted between two chambers containing 15 ml each of the Ringer's solution. The potential across the bladder was monitored by two calomel half-cells and reduced to zero by a voltage clamp. The test peptide was added to the serosal medium. At least 10 min were allowed for the response to develop. The intensity of the "short-circuit" current which indicates the rate of sodium transport across the bladder was recorded.

A 2 × 2 assay design (18) was employed in both the hydro-osmotic and the natriferic assays. Synthetic oxytocin, Syntocinon (Sandoz) was used as the reference standard.

The six cyclic pentapeptides studied in this report were all generously supplied by Professor Vincent du Vigneaud of Cornell University. They were pressinoic acid, pressinamide, tocinamide and their deamino analogs, deaminopressinoic acid, deaminopressinamide and deaminotocinamide. The oxytocin used was either Pitocin (Parke-Davis) or Syntocinon (Sandoz).

Results. The six cyclic pentapeptides studied in this report all correspond to either the ring structure of vasopressin or oxytocin. Pressinoic acid corresponds to the ring structure of vasopressin with a terminal carboxyl group. Pressinamide and tocinamide correspond to the ring structures of vasopressin and oxytocin, respectively, and both have a terminal carboxamide group. Their chemical structures are shown in Fig. 1. The deamino analogs, deaminopressinoic acid, deaminopressinamide and deaminotocinamide differ from their respective parent molecule only in that the N-terminal amino group is replaced by hydrogen.

Antidiuretic and natriuretic activities. Each pentapeptide was tested in three or more rats for antidiuretic and natriuretic activities. The average values from these experiments are shown in Table I. The antidiuretic activity was assayed against the USP Posterior Pituitary Reference Standard. It is clear that the ring structures, in contrast to the hormones vasopressin and oxytocin, possess little or no antidiuretic activity.

Natriuretic activity is difficult to quantitate. Nevertheless, a relative comparison revealed that the oxytocin rings consistently had a higher natriuretic activity than the

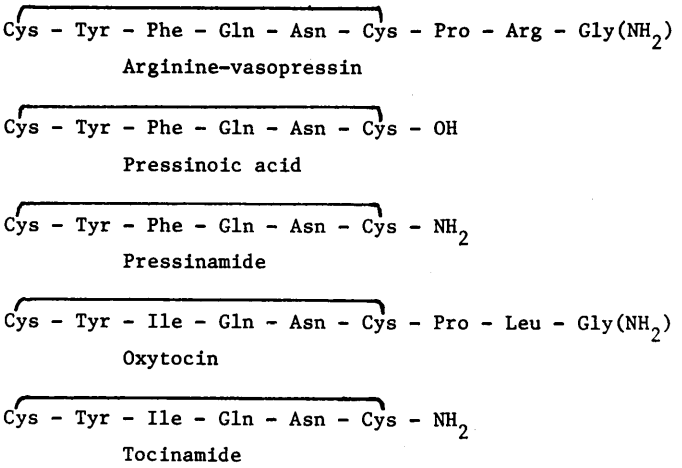

FIG. 1. Chemical structures of the pentapeptide rings of neurohypophysial hormones. Structures of the deamino analogs are not shown. The deamino analogs differ from their respective parent molecules only by the absence of their N-terminal amino groups. In the deamino analogs, the free amino groups are replaced by hydrogen.

TABLE I. Natriuretic and Antidiuretic Activities of the Ring Structures of Neurohypophysial Hormones in Rats.[a]

	Urinary sodium excretion rate							
	Preinjection			Postinjection				Antidiuretic activity
	U_1	U_2	U_3	U_4	U_5	U_6	U_7	(U/mg)
Pressinoic acid	98	96	100	96	116	94	85	~0.03
Deaminopressinoic acid	85	99	100	118	187	183	144	~0.6
Pressinamide	109	108	100	110	115	210	181	~0.5
Deaminopressinamide	120	99	100	50	111	206	178	~1.0
Tocinamide	110	128	100	271	223	121	114	<0.01
Deaminotocinamide	129	118	100	106	353	493	319	~0.01
Oxytocin[b]	98	99	100	390	580	370	330	3.0
Lysine–vasopressin								200

[a] All urine samples were collected at 3 min intervals. The cyclic pentapeptides were tested at a fixed dose of 0.7 μg/100 g. The sodium excretion rates immediately prior to the injections are taken as 100%. All other excretion rates are expressed as percentage of this control value. The low value of U_4 for deaminopressinamide was an artifact resulting from a dead-space error due to the marked antidiuresis induced by the pentapeptide. The values shown are the average values of three or more experiments.

[b] Because of the relative high antidiuretic activity of oxytocin, an equivalent dose could not be injected. The dose of oxytocin injected was a strong antidiuretic dose, 0.05 mU (antidiuretic activity)/100 g = 0.016 μg/100 g.

vasopressin rings. Deaminotocinamide had marked natriuretic activity, although this was still far less than that of oxytocin (Table I).

Hydro-osmotic and natriferric activities. The hydro-osmotic and natriferic activities of the pentapeptides were assayed in the toad urinary bladder. Four determinations were done on each peptide. The average values of these experiments are shown in Table II. The

TABLE II. Hydro-osmotic and Natriferic Activities on the Toad Bladder.[a]

	Activity (U/mg)	
	Hydro-osmotic	Natriferic
Pressinoic acid	~0.70	~1.17
Deaminopressinoic acid	~0.10	~0.07
Pressinamide	3.84	~6.63
Deaminopressinamide	1.50	~3.38
Tocinamide	232	~320
Deaminotocinamide	130	~141
Oxytocin[b]	450	450

[a] Each value shown is the average value of four determinations.

[b] Oxytocin (Syntocinon) used as reference standard.

vasopressin rings were found to have little hydro-osmotic or natriferic activity in the toad-bladder. The oxytocin rings on the other hand retained very high hydro-osmotic and natriferic activities. The high potency of tocinamide was remarkable. In a few experiments, it was observed that the maximal hydro-osmotic and natriferic responses induced by tocinamide appeared comparable to the maximal responses induced by oxytocin. Figure 2 shows the natriferic responses to oxytocin and tocinamide as measured by the "short-circuit" current method.

Discussion. A number of cyclic pentapeptides corresponding to the ring structures of oxytocin and vasopressin have recently been synthesized by du Vigneaud and his associates (12–14). These investigators also studied the biological activities of these peptides and found that they possessed little or no vasopressor, avian depressor and oxytocic activities which are characteristic of the neurohypophysial hormones. Only tocinamide and deaminotocinamide were found to retain weak but significant oxytocic activity.

Our interest here was to assess the impor-

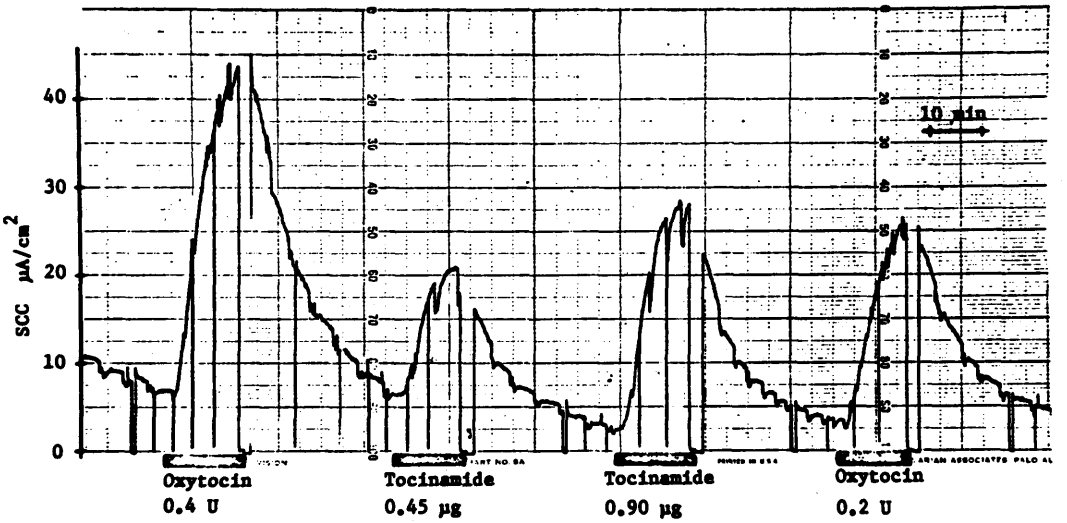

FIG. 2. Natriferic responses to oxytocin and tocinamide in a toad bladder. The rate of sodium transport across the bladder was measured by the "short-circuit" current (SCC). The shaded horizontal bars indicate the periods when test peptides were in the serosal medium. The serosal bath volume was 15 ml.

tance of the ring component of oxytocin and vasopressin to the effects of the hormone on water and sodium transport.

Our assays on rat antidiuretic activity show clearly that all six cyclic pentapeptides had detectable antidiuretic activity, although the level of activity was extremely low compared to the natural hormones. It is interesting to note that the four vasopressin rings had a higher antidiuretic activity than the oxytocin rings. The vasopressin ring with a terminal carboxamide group was more potent than the vasopressin ring with a terminal carboxyl group. The effect of replacing the N-terminal amino group is of particular interest. It has been shown that replacement of the free amino group in oxytocin and vasopressin with hydrogen enhances the oxytocic and the antidiuretic activities (6, 19). The same effect was found with the ring structures. The deamino analogs had a higher oxytocic potency (12–14) and a higher antidiuretic potency (Table I).

Because of the limited supply of material, the determination of natriuretic activity was rather restricted. The relative natriuretic activity was compared in rats at a fixed dose of 0.7 μg/100 g. At this dose level, pressinoic acid had no demonstrable natriuretic activity. Deaminopressinoic acid, pressinamide and deaminopressinamide had slight natriuretic activity of about the same magnitude. At the peak of action, each approximately doubled the urinary excretion rate of sodium. The natriuretic activity of the oxytocin rings was higher. Tocinamide and deaminotocinamide increased the urinary excretion rate of sodium three times and four times, respectively. Although the natriuretic activity of the oxytocin rings represents only a fraction of that of oxytocin, nevertheless, it is clear from our experiments that the ring moiety of oxytocin retains a high degree of natriuretic activity.

All six cyclic pentapeptides at appropriate dosage produced the typical hydro-osmotic and natriferic responses in the toad bladder. The activities were, however, reduced when compared to that of oxytocin and vasopressin. Again, as was found in the rat kidney, the carboxamide ring of vasopressin was more active than the carboxyl ring of vasopressin. Replacement of the free amino group in the ring structure with hydrogen produced a similar change in activity as that observed in deaminooxytocin and deaminovasopressin. The deamino analogs consistently had a lower hydro-osmotic and natriferic activity than their respective parent molecules. The po-

tency of tocinamide was remarkable and was nearly comparable to that of oxytocin. The dose–response curve of tocinamide was similar to that of oxytocin. Furthermore, it exhibited a similar maximal response. Thus our experiments indicate that the intrinsic hydro-osmotic and natriferic activities of oxytocin reside primarily in the ring moiety. This is consistent with earlier observations that the unaltered oxytocin ring structure is highly important in determining the hydro-osmotic and natriferic activities of neurohypophysial peptides.

Our data also appear consistent with the finding of Morel and Bastide (20) that oxytocin analogs in which glycine or leucine were deleted from the side chain had greater natriferic activity on frog skin than oxytocin itself. Our estimate of the activity of tocinamide on toad bladders does not agree with that of Rasmussen *et al.* (21) who reported that the oxytocin ring amide had less than 1% of the hydro-osmotic activity of oxytocin. The reasons for this apparent discrepancy are not clear.

Summary. Six cyclic pentapeptides corresponding to the ring structures of oxytocin and vasopressin were studied for water and sodium transport activities in the rat kidney and the toad urinary bladder. All six cyclic pentapeptides had detectable antidiuretic activity in the rat, although the level of activity was extremely low compared to the natural hormones. They also had natriuretic activity. The natriuretic activity of the oxytocin rings was higher than that of the vasopressin rings. All six cyclic pentapeptides at appropriate dosage produced the typical hydro-osmotic (water permeability) and natriferic (sodium transport) responses in the toad bladder. The activities for the vasopressin rings were low. The oxytocin rings on the other hand retained very high hydro-osmotic and natriferic activities. Our data thus indicate that the intrinsic natriuretic, hydro-osmotic and natriferic activities of oxytocin reside primarily in the ring moiety.

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1. Sawyer, W. H., Amer. J. Physiol. **169**, 583 (1952).

2. Anslow, W. P., Jr., and Wesson, L. G., Amer. J. Physiol. **182**, 561 (1955).

3. Croxatto, H., Rosa, R., and Barnafi, L., Acta Physiol. Lat. Amer. **6**, 147 (1956).

4. Brooks, F. P., and Pickford, M., J. Physiol. (London) **142**, 468 (1958).

5. Chan, W. Y., and Sawyer, W. H., Amer. J. Physiol. **201**, 799 (1961).

6. Chan, W. Y., Endocrinology **77**, 1097 (1965).

7. Sawyer, W. H., and Schisgall, R. M., Amer. J. Physiol. **187**, 312, (1956).

8. Bentley, P. J., J. Endocrinol. **17**, 201 (1958).

9. Leaf, A., Anderson, J., and Page, L. B., J. Gen. Physiol. **41**, 657 (1958).

10. Sawyer, W. H., Pharmacol. Rev. **13**, 225 (1961).

11. Pickering, B. T., *in* "Pharmacology of the Endocrine System and Related Drugs: The Neurohypophysis" (H. Heller and B. T. Pickering, eds.), p. 81. Pergamon, New York (1970).

12. Hruby, V. J., Ferger, M. F., and du Vigneaud, V., J. Amer. Chem. Soc. **93**, 5539 (1971).

13. Ferger, M. F., Jones, W. C., Dyckes, D. F., and du Vigneaud, V., J. Amer. Chem. Soc. **94**, 982 (1972).

14. Hruby, V. J., Smith, C. W., Linn, D. K., Ferger, M. F., and du Vigneaud, V., J. Amer. Chem. Soc. **94**, 5478 (1972).

15. Sawyer, W. H., Methods Med. Res. **9**, 210 (1961).

16. Sawyer, W. H., Endocrinology **66**, 112 (1960).

17. Ussing, H. H., and Zerahn, K., Acta Physiol. Scand. **23**, 110 (1950).

18. Schild, H. O., J. Physiol. (London) **101**, 115 (1942).

19. Chan, W. Y., and du Vigneaud, V., Endocrinology **71**, 977 (1962).

20. Morel, F., and Bastide, F., *in* "Oxytocin, Vasopressin and their Structural Analogues" (J. Rudinger, ed.), p. 47. Pergamon, New York (1964).

21. Rasmussen, H., Schwartz, I. L., Young, R., and Marc-Aurele, J., J. Gen. Physiol. **46**, 1171 (1963).

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