insight than we have at present regarding the structure-function relationships in androgen molecules.

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Solid-Phase Synthesis and Some Pharmacological Properties of Deamino-4-threonine Analogs of the Vasopressins and Vasotocin and [Deamino]arginine-vasotocin†

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[Deamino,4-threonine]arginine-vasopressin (I), [deamino,4-threonine]lysine-vasopressin (II), [deamino,4-threonine]arginine-vasotocin (III), and [deamino]arginine-vasotocin (IV) were synthesized by the solid-phase method and tested for their biological activities. Compounds I–III exhibited a general reduction in rat uterus, fowl vasodepressor, and rat vasopressor potencies but possessed a selective enhancement of antidiuretic potencies when compared to the parent [1-amino-4-threonine] analog in each case. Thus, I–III exhibited diminishments of 45, 80, and 70% in rat uterus activity; 40, 27, and 5% in fowl vasodepressor activity; 70, 80, and 70% in rat vasopressor activity. I–III exhibited enhancements of 230, 250, and 25% in antidiuretic activity. This selectivity has resulted in the following antidiuretic to pressor ratios: I, 25; II, 54; and III, 11. The corresponding ratios in arginine-vasopressin, lysine-vasopressin, and arginine-vasotocin are 0.9, 1.1, and 1.4. Upon bioassays, IV was found to possess 251 ± 12 units/mg of rat uterus activity, 1174 ± 24 units/mg of fowl vasodepressor activity, 890 ± 100 units/mg of antidiuretic activity, and 256 ± 6 units/mg of rat vasopressor activity. These represent increases of 100, 138, 280, and 60% over the corresponding values for arginine-vasotocin.

In a preceding paper[2] we have shown that substitution of the glutamine residue in position 4 of the basic neurohypophysial peptides by a threonine residue resulted in a series of analogs exhibiting on the one hand a selective enhancement of oxytocin-like characteristics and on the other hand a selective diminishment of vasopressin-like characteristics, while at the same time giving rise to an increase in the ratio of antidiuretic to rat pressor activities in each of the three analogs studied.

It was speculated that an enhancement of the overall lipophilicity of each 4-threonine-substituted molecule as compared to the parent molecule might contribute to the observed antidiuretic-pressor selectivity. Earlier experiments had shown that the removal of the amino group from the [4-threonine] analogs of oxytocin and mesotocin had given rise to deamino analogs possessing greatly enhanced lipophilic properties.[3] It was thus considered worthwhile to try to explore this speculation further by preparing the analogous [deamino,4-threonine] analogs of the basic neurohypophysial peptides, *i.e.*, [deamino,4-threonine]arginine-vasopressin, [deamino,4-threonine]lysine-vasopressin, and [deamino,4-threonine]arginine-vasotocin. The present investigation was further prompted by the surprising properties exhibited by both [deamino,4-threonine]oxytocin and [deamino,4-threonine]mesotocin.[3] Instead of possessing the enhancement of activities which markedly characterized previously prepared deamino oxytocin analogs,[4-8] both analogs were found to possess markedly diminished potencies, by comparison with the parent [4-threonine] analog in each case, in all of the characteristic assay systems. The present investigation was therefore also undertaken to determine whether or not removal of the amino group from the 4-threonine analogs of the basic neurohypophysial peptides would bring about these same diminished effects[3] or an enhancement similar to that observed upon deamination of arginine-vasopressin[9] and lysine-vasopressin.[10]

The synthesis of [deamino]arginine-vasotocin has recently

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Table I. Biological Activities of the Vasopressins and Vasotocin, Their 4-Threonine, Deamino, and Deamino-4-threonine Analogs

Analog	Biological activities (U/mg ± S.E. of the assays)					Ratio Antidiuretic:vaso-depressor
	Rat uterus					
	No Mg^{2+}	0.5 m*M* Mg^{2+}	Fowl depressor	Antidiuretic	Vasopressor	
Arginine-vasopressin[a] (AVP)	13.9 ± 0.5	25.5 ± 0.6	105 ± 5	323 ± 16	369 ± 6	0.9
Deamino-AVP[b]	47 ± 3	66 ± 3	150 ± 4	1390 ± 136	(370 ± 20)	3.8
Thr^4-AVP[a]	18.6 ± 0.4	65 ± 3	321 ± 15	231 ± 29	104 ± 2	2.2
Deamino-Thr^4-AVP[e]	10.2 ± 0.5	19.7 ± 1.8	195 ± 9	758 ± 50	30 ± 1	25
Lysine-vasopressin[c] (LVP)	10.1 ± 0.3	22.1 ± 0.9	52 ± 6	284 ± 39	(270 ± 15)	1.1
Deamino-LVP[d]	12 ± 0.5		61 ± 2	301 ± 11	126 ± 2	2.4
Thr^4-LVP[a]	12.9 ± 0.4	25.1 ± 0.8	267 ± 5	155 ± 17	49 ± 2	3.2
Deamino-Thr^4-LVP[e]	2.7 ± 0.2	7.5 ± 0.3	195 ± 9	544 ± 62	10 ± 0.4	54
Arginine-vasotocin[a] (AVT)	127 ± 9	194 ± 15	493 ± 17	231 ± 30	160 ± 4	1.4
Deamino-AVT[e]	251 ± 12	206 ± 10	1174 ± 24	890 ± 100	256 ± 6	3.5
Thr^4-AVT[e]	201 ± 10	260 ±21	831 ± 27	279 ± 25	106 ± 25	2.6
Deamino-Thr^4-AVT[e]	63 ± 2	82 ± 3	786 ± 40	348 ± 33	33 ± 1	11

[a]Values are those reported by Manning, *et al.*[2] [b]Assays done on a solution of the peptide prepared by Huguenin and Boissonnas.[9] The absolute activity in vasopressor units was assumed to be the same as that they reported. The remaining activities were calculated from our assays on this assumption. [c]Assays on a solution of Sandoz synthetic lysine-vasopressin. The absolute pressor activity was assumed to be that reported by B. Berde and R. A. Boissonnas in "Neurohypophysial Hormones and Similar Polypeptides," B. Berde, Ed., Springer-Verlag, Berlin, 1968, p 802. [d]Values are those reported by Kimbrough, *et al.*[10] [e]All assays were done by the authors and, except where indicated, the values shown represent units/mg of lyophilized peptides synthesized by the authors.

Table II. Rat Antidiuretic and Bull Frog Bladder Hydroosmotic Activities of [8-Arginine]vasotocin and Oxytocin, Their [4-Threonine], [Deamino], and [Deamino,4-threonine] Analogs

Peptide	Activities, U/mg[a]		Activity ratio Hydroosmotic / antidiuretic
	Rat, antidiuretic	Bull frog bladder, hydroosmotic	
Arginine-vasotocin (AVT)	231 ± 30	59,000 ± 7900	255
Deamino-AVT	890 ± 100	35,000 ± 3100	39
Thr^4-AVT	279 ± 25	3,240 ± 280	12
Deamino-Thr^4-AVT	348 ± 33	415 ± 34	1.2
Oxytocin	4 ± 0.8	520	130
Deamino-oxytocin	19	72	4
Thr^4-oxytocin	1.8 ± 0.3	13	7

[a]Antidiuretic activities estimated by assays against USP reference standard. For hydroosmotic assays oxytocin was used as standard. One unit of hydroosmotic activity is equivalent to the activity of 1 USP unit of oxytocin.

been reported[11] but details of its pharmacological properties were not included. Knowledge of these properties was felt to be essential to this study. Thus the synthesis and pharmacological investigation of this compound are also included in this report. All syntheses were carried out as described in the Experimental Section. The pharmacological properties were studied by methods previously described.[12]

Results and Discussion

The data in Table I present an interesting picture of the different effects brought about upon removal of the amino group from the 4-glutamine-containing peptides on the one hand and the 4-threonine-containing ones on the other. It can be seen that [deamino]arginine-vasotocin follows the same pattern as that found earlier for both [deamino]arginine-vasopressin[9] and [deamino]lysine-vasopressin;[10] *i.e.*, there is an increase in both the oxytocin-like and vasopressin-like activities compared to those of arginine-vasotocin. By contrast, removal of the amino group from each of the 4-threonine derivatives of the vasopressins and vasotocin has resulted in deamino analogs possessing diminished potencies in the rat uterus, fowl vasodepressor, and rat vasopressor assay systems. Thus, this effect is analogous to that observed upon removal of the amino groups of the 4-threonine analogs of the neutral oxytocin and mesotocin peptides.[3] However, unlike these neutral peptides, in which all of the characteristic potencies were diminished, deamination of the 4-threonine analogs of the basic peptides resulted in a curious dichotomy of effects. For it will be noted from Table I that in all three analogs there is a substantial enhancement of the antidiuretic activities as compared to each parent 4-threonine-substituted analog. This enhancement of antidiuretic potency parallels that previously observed for deamination of the natural 4-glutamine-containing basic peptides.[9,10] Furthermore, these diverse effects on the pressor and antidiuretic activites have brought about very substantial increases in the ratio of antidiuretic to pressor activities for all three deamino 4-threonine analogs. This would tend to support the speculation mentioned above that increasing the overall lipophilicity of the molecules is a key factor involved. These findings bear a striking similarity to those found earlier by Gillessen and du Vigneaud[13,14] for the removal of the amino group from arginine- and lysine-vasopressin analogs containing α-aminobutyric acid at position 4. Both deamino analogs were shown to possess dramatically high antidiuretic to pressor ratios: 95 and 200 for the [deamino,4-α-aminobutyric acid]arginine-vasopressin and [deamino,4-α-aminobutyric acid]lysine-vasopressin analogs, respectively.

The diminishment of the bull frog bladder hydroosmotic activity of arginine-vasotocin brought about by increasing the lipophilicity of the molecule is indicated in Table II. Thus, [deamino,4-threonine]arginine-vasotocin is even less potent than oxytocin. In regard to the peptide-receptor interactions involved, it would now appear, on the basis of these and other studies,[13,14] that the receptors which mediate the antidiuretic response are of a more general lipophilic nature than those which mediate either the pressor or the bull frog bladder hydroosmotic response. This information should aid in the design and study of analogs possessing even greater selected antidiuretic properties for clinical use in those situations in which treatment with lysine-vasopressin or arginine-vasopressin is currently indicated.

Experimental Section‡

The required protected octapeptides **1**, **3**, **5**, and **7** were all synthesized with the use of the solid-phase method.[15,16] Manual

‡For quantitative amino acid analyses, samples were hydrolyzed with constant boiling HCl in evacuated sealed ampoules at 110° for 18 hr and analyzed on a Beckman/Spinco amino acid analyzer Model 121C according to the method of Spackman, *et al.*[19] All optical rotations were measured on a Bellingham Stanley Ltd., Model A polarimeter, Type P1.

Table III. Protected β-Mercaptopropionyl Octapeptides of [Deamino,4-threonine]arginine-vasopressin (1), [Deamino,4-threonine]arginine-vasotocin (3), [Deamino,4-threonine]lysine-vasopressin (**5**), and [Deamino]arginine-vasotocin (7)

Bzl Bzl Bzl
S-$(CH_2)_2$CO-Tyr-(X)-(X)-Asn-Cys-Pro-(X)-Gly-NH_2
1 2 3 4 5 6 7 8 9

No.	Amino acids in position 3	4	8	Formula[a]	Mp,[b] °C	$[\alpha]^T D$,[c] deg	Yield, %, on resin[d]	Yield, %, on ammonolysis[d]
1	Phe	Thr(Bzl)	Arg(Tos)	$C_{80}H_{95}N_{13}O_{14}S_3 \cdot 2H_2O$	216–218	−15.4	65.0	42.5
3	Ile	Thr(Bzl)	Arg(Tos)	$C_{77}H_{97}N_{13}O_{14}S_3$	235–236	−16.5	100	71
5	Phe	Thr(Bzl)	Lys(Tos)	$C_{80}H_{95}N_{11}O_{14}S_3$	215–216	−17.4	98	80
7	Ile	Gln	Arg(Tos)	$C_{71}H_{92}N_{14}O_{14}S_3$	228–230	−29.1	82	50

[a] Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn. The analytical results were within ±0.4% of the theoretical values; all compounds were analyzed for C, H, and N. [b] Melting ponts were taken in an open capillary in a Thomas-Hoover melting point apparatus and are uncorrected. [c] In DMF (*c* 1.0), *T* = 21, 20, 22, and 22.5°, respectively. [d] Yields are based on the initial glycine incorporation on the resin.

Table IV. [Deamino,4-threonine]-8-arginine-vasopressin (2), [Deamino,4-threonine]-8-arginine-vasotocin (4), [Deamino,4-threonine]-8-lysine-vasopressin (**6**), and [Deamino]-8-arginine-vasotocin (8)

S-$(CH_2)_2$CO-Tyr-(X)-(X)-Asn-Cys-Pro-(X)-Gly-NH_2
1 2 3 4 5 6 7 8 9

No.	Amino acids in positions 3	4	8	$[\alpha]^T D$,[a] deg	R_f[b]	*E*(Lys),[c] A	*E*(Lys),[c] B	Yield, %, from protected nonapeptide	Yield, %,[d] overall
2	Phe	Thr	Arg	−204.5	0.24	0.32	0.18	30.0	12.6
4	Ile	Thr	Arg	−6.7	0.24	0.32	0.18	40.5	28.0
6	Phe	Thr	Lys	−101.2	0.20	0.32	0.18	45.3	36.0
8	Ile	Gln	Arg	−81.2	0.20	0.32	0.18	43.4	21.7

[a] In 1 *N* acetic acid (*c* 0.5), *T* = 22.0, 19.0, 22.0, and 23.0°, respectively. [b] Samples run on silica gel H plates in the upper phase of the solvent system *n*-BuOH–AcOH–H_2O (4:1:5, v/v, ascending).[18] [c] Electrophoresis in two pyridine acetate buffers of pH 3.5 (A) and 6.5 (B) gave only one spot with the platinum reagent in all cases.[18] Lysine was used as a reference compound and *E*(Lys) indicates the electrophoretic mobility relative to lysine = 1.00 in each of the buffer systems A and B. [d] Based on the initial glycine incorporation on the resin.

or automated methods following procedures previously described,[3,17,18] and with the changes noted below, were used.

***S*-Bzl-β-mercaptopropionyl-Tyr(Bzl)-Phe-Thr(Bzl)-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (1).** Boc-glycyl resin (2.38 g, 0.875 mmol of glycine) was treated in an eight-cycle procedure as described for the synthesis of [deamino,4-threonine]oxytocin[3] except that Boc-Arg(Tos) (in DMF) and Boc-Phe were used in the first and sixth incorporation steps, respectively, to give the protected resin, 3.18 g. Ammonolytic cleavage of the protected octapeptide resin (2.45 g) was carried out as described earlier[17,18] and the protected peptide was extracted with DMF and MeOH. Solvents were removed *in vacuo* and the residue was purified by trituration with MeOH (20 ml) to give 1 as an amorphous white powder, wt 450 mg (Table III). Amino acid analysis[19] gave Asp, 1.02; Thr, 0.90; Gly, 1.00; Bzl-Cys, 0.90; Phe, 0.98; Tyr, 0.67; Pro, 0.98; Arg, 0.91; NH_3, 1.92.

[Deamino,4-threonine]arginine-vasopressin (2). The protected octapeptide 1 (150 mg) was deblocked by the sodium–liquid ammonia procedure[20] as used in the original synthesis of oxytocin.[21,22] Reoxidation in aqueous solution at pH 6.5 was effected with the use of potassium ferricyanide.[4b] The lyophilized product was purified§ by gel filtration on Sephadex G-15 by the previously described[23] two-step procedure involving sequential elution with 50% AcOH and 0.2 *N* AcOH, as modified for the purification of [deamino,4-threonine]-oxytocin.[3] [Deamino,4-threonine]arginine-vasopressin obtained as a fluffy white powder, wt 30.0 mg, was shown to be homogeneous by thin-layer chromatography and paper electrophoresis at different pH's as described for [4-threonine]oxytocin[18] (Table IV). Amino acid analysis[19] gave Asp, 0.98; Thr, 0.95; Gly, 1.00; Pro, 0.97; Phe, 0.95; Tyr, 0.85; Arg, 0.96; NH_3, 2.32. In addition, cystine (0.39) and the mixed disulfide of cysteine and β-mercaptopropionic acid (0.47) were present.

***S*-Bzl-β-mercaptopropionyl-Tyr(Bzl)-Ile-Thr(Bzl)-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (3).** Boc-glycyl resin (3.40 g, 1.26 mmol of glycine) was treated as described for the synthesis of [deamino,4-threonine]oxytocin[3] except that Boc-L-Arg(Tos) (in DMF) was used in the first incorporation step. Upon completion of the eight-cycle procedure the protected octapeptide resin was collected and dried *in vacuo* over P_2O_5, wt 5.1 g. Ammonolytic cleavage[17,18] of the protected nonapeptide resin (2.3 g) followed by trituration with MeOH (20 ml) gave 3 as a white amorphous powder, wt 620 mg (Table III) Amino acid analysis[19] gave Asp, 1.00; Thr, 0.89; Pro, 0.91; Gly, 1.00; Ile, 1.01; Tyr, 0.81; Bzl-Cys. 0.96; Arg, 0.99; NH_3, 2.08.

[Deamino,4-threonine]arginine-vasotocin (4). The protected octapeptide 3 (150 mg) was reduced, reoxidized, deionized, lyophilized, and purified as for 1 above, wt 40.0 mg. It was shown to be homogeneous by thin-layer chromatography and paper electrophoresis at two different pH's (Table II). Amino acid analysis[19] gave Asp, 0.97; Thr, 0.88; Pro, 0.98; Gly, 1.00; Ile, 0.87; Tyr, 0.97; Arg, 0.90; NH_3, 1.93; cystine 0.42; mixed disulfide of cysteine and β-mercaptopropionic acid, 0.47.

***S*-Bzl-β-mercaptopropionyl-Tyr(Bzl)-Phe-Thr(Bzl)-Asn-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2 (5).** The protected octapeptide resin was prepared from Boc-glycyl resin (4.45 g, 1.78 mmol of glycine) with the use of an automated machine (purchased from Schwartz Bioresearch, Inc.) by the method used for the synthesis of [deamino,4-threonine]oxytocin[3] except that *N*-Boc-L-Lys(Tos) and Boc-L-Phe were used in the first and sixth incorporation steps, respectively, wt 6.97 g. Ammonolytic cleavage[17,18] of this octapeptide resin (1.5 g) yielded **5**. This material was very insoluble in MeOH and was therefore readily purified by simply triturating and washing with this solvent, wt 470 mg (Table III). Amino acid analysis[19] gave Asp, 1.03; Thr, 0.93; Gly, 1.00; Bzl-Cys, 1.00; Phe, 1.01; Tyr, 0.71; Pro, 1.01; Lys, 0.81; NH_3, 2.24.

[Deamino,4-threonine]lysine-vasopressin (6). The protected octapeptide **5** (150 mg) was deblocked, reoxidized, and purified as for 1, wt 45.0 mg (Table IV). It was shown to be homogeneous by the usual methods.[18] Amino acid analysis[19] gave Asp, 1.02; Thr, 0.95; Gly, 1.00; Pro, 1.01; Phe, 0.99; Tyr, 0.86; Lys, 0.94; NH_3, 2.20; cystine 0.22; mixed disulfide, 0.68.

***S*-Bzl-β-mercaptopropionyl-Tyr(Bzl)-Ile-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (7).** Boc-glycyl resin (5.0 g, 2.0 mmol of glycine) was treated by the eight-cycle procedure used for the synthesis of [8-phenylalanine]oxytocin[24] except that in this instance an automated machine was used. Boc-Arg(Tos) and *S*-benzyl-β-mercaptopropionic acid as its *p*-nitrophenyl ester[4] were utilized in the first and last incorporations. Both derivatives were coupled in redistilled DMF. The protected octapeptide resin was collected in the usual manner,[24] wt 7.11 g. Ammonolysis[17,18] of the protected octa-

§ In contrast to the problems encountered in trying to solubilize the deamino derivatives of both [4-threonine]oxytocin and [4-threonine]mesotocin in 0.2 *N* AcOH,[3] all the deamino analogs presented in this report were found to be very readily soluble in this solvent.

peptide resin (2.5 g) gave **7** as an amorphous powder. It was purified as for **5**, wt 550 mg (Table III). Amino acid analysis[19] gave Asp, 1.05; Glu, 1.02; Gly, 1.00; Bzl-Cys, 0.97; Ile, 0.89; Tyr, 0.81; Pro, 0.97; Arg, 0.91; NH_3, 2.94.

[Deamino,8-arginine]vasotocin (8). Reduction, reoxidation, and purification of **7** (150 mg) as for 1 gave **8** as a white fluffy powder, wt 46 mg, shown to be homogeneous by thin-layer chromatography and by electrophoresis.[18] Amino acid analysis[19] gave Asp, 1.03; Gly, 0.97; Glu, 1.05; Pro, 0.90; Tyr, 0.91; Ile, 0.92; Arg, 1.00; NH_3, 2.90; cystine, 0.40; mixed disulfide, 0.51.

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3-(16β,17β-Dihydroxy-3-oxoandrost-4-en-17α-yl)propionic Acid γ-Lactone, Its Preparation and Antimineralocorticoid Activity†

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The title compound was prepared in which dehydroisoandrosterone was employed as the starting material. The 16β-hydroxyl group was introduced by lead tetraacetate oxidation and was protected as the acetonide during the construction of the spirolactone ring. The antimineralocorticoid potency of the final product was less than 13.8% that of spironolactone.

Aldosterone **(1)** is a potent mineralocorticoid which plays an important role in regulating the electrolyte composition of the body fluid. In promoting the excretion of potassium and the retention of sodium ions, it gives rise to the edematous state that is often seen in patients afflicted with congestive heart failure, nephrosis, or cirrhosis of the liver.[1]

A rational approach to the treatment of these patients would be to block the activity of aldosterone and related mineralocorticoids, such as deoxycorticosterone **(2a)**. Since some steroids having a spirolactone side chain at C-17, *e.g.*, **3a**, have been found to be aldosterone blockers,[2] attempts have been made to modify their structures in the hope of obtaining compounds which would be even more potent.[3]

A structural feature that is common to both aldosterone and the spirolactones is the oxaspiran unit in which a tetrahydrofuran ring is fused to ring D. In aldosterone the spiro atom is C-13, while in the spirolactones it is C-17.[4]

The possibility that an oxaspiran unit is involved in some manner with the transport of Na^+ and K^+ across cellular membranes is purely speculative, but it is an intriguing one and it has been extensively explored in the search for compounds having a diuretic effect.[3]

Metabolic studies[5] offer another source from which new leads can be generated. Conceivably, a metabolic product of the administered compound is the active species. Hydroxylation is one means by which compounds are metabolized *in vivo*. Because of the ease with which a hydroxyl group can be introduced into a molecule by microorganisms, numerous spirolactones having a hydroxyl group at various positions in the molecule have been prepared and tested for their antimineralocorticoid effects.[3b,6] To date, however, there has been no report of the preparation of the spirolactone in which a hydroxyl group is located at the 16β position **(3b)**. In view of the claims made that certain 16-hydroxylated steroids possess sodium-excreting properties,[7] the effect which **3b** exerts on the mineralocorticoids should be of interest.

Since cardiac steroids are known to affect electrolyte transport,[8] a recent report that 16-acetylgitoxin **(4)** produced favorable cardiotonic effects[9] prompts us to report the synthesis of the 16β-hydroxyspirolactone **(3b)** and the effect which it displayed in our antimineralocorticoid test.

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