administered vehicle control solution. Four rats per dose were tested and each rat's performance under the drug treatment was compared to his previous performance under vehicle control treatment. Comparisons were made by means of a paired t test ($p \leq 0.05$, two tailed). Usually three doses of a compound were evaluated. Most drugs were evaluated 30 min postinjection but intervals of 1–90 min could be used.

Receptor Binding Studies. Calf caudate nuclei were dissected from freshly obtained brains and stored frozen at −76 °C. As needed, caudate tissue was homogenized and prepared following procedures outlined by Creese and co-workers.[11]

Receptor binding studies were performed as reported in the literature[11,13,15] with slight modifications. A typical sample contained 2 mL of caudate membrane homogenate (10 mg of original tissue/mL) in a final ligand concentration of either 5 nM [^{3}H]dopamine or 1.6 nM [^{3}H]haloperidol. Test compounds were added as 20-μL aliquots from stock solutions prepared in absolute ethanol or 0.1% ascorbic acid. Samples were incubated in triplicate at 37 °C for 15 min when [^{3}H]dopamine was used and for 10 min when [^{3}H]haloperidol was present.

Immediately following all incubations, proteins were recovered on Whatman GF/B glass fiber filters under reduced pressure. Trapped membranes were solubilized off the filters using 1 mL of NCS tissue solubilizer (Amersham/Searle Corp.) at 50 °C for 1 h. Then the pH was adjusted by adding 0.1 mL of glacial acetic acid, 10 mL of PCS (Amersham/Searle Corp.) was added, and the samples were analyzed for membrane-bound radioactivity using a Mark II liquid scintillation counter (Searle Analytical, Inc.).

Nonspecific binding was measured in the presence of 10^{-5} M (+)-butaclamol for the [^{3}H]dopamine studies and 10^{-4} M nonradiolabeled dopamine for the [^{3}H]haloperidol studies. IC_{50} values were determined from log probit using four to six concentrations of each compound.

Acknowledgment. Appreciation is extended to Mr. J. Bloss for in vivo biological assays, Mr. E. Zielinski for microanalytical results, Mr. R. Ray for chemical assistance, Dr. F. Hershenson for useful discussions, and Ms. D. Sheller and Ms. J. Vassilatos for preparation of the manuscript.

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Synthesis of O-Alkylated Lysine-vasopressins, Inhibitors of the Antidiuretic Response to Lysine-vasopressin

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[Mpa^1,Tyr(Et)^2]-LVP (1-deamino-2-*O*-ethyltyrosine-8-lysine-vasopressin), [Mpa^1,Tyr(*n*-Pr)^2]-LVP, [Tyr(*n*-Bu)^2]-LVP, [Mpa^1,Tyr(*n*-Bu)^2]-LVP, and [Mpa^1,Tyr(*n*-hexyl)^2]-LVP were synthesized in solution by the *p*-nitrophenyl ester method. The previously prepared [Tyr(Et)^2]-LVP was resynthesized. All compounds possessed weak agonistic properties in both antidiuretic (0.5–2.0 IU/μmol) and pressor (0.5–3.0 IU/μmol) assays. In the rat none of the analogues inhibited the antidiuretic action of LVP when the two substances were given together in a single injection. However, when administered in low subthreshold doses, most of the deamino compounds suppressed the antidiuresis induced by a continuous infusion of LVP. Complete inhibition was obtained with [Mpa^1,Tyr(Et)^2]-LVP. The antagonistic potency seemed to decrease with increasing size of the alkyl substituent and [Mpa^1,Tyr(*n*-hexyl)^2]-LVP showed no antagonism. The molar inhibitor–LVP ratio for maximal inhibition was well below 100. Neither of the two amino analogues showed a clear-cut antagonism in the antidiuretic assay. Furthermore, none of the reported compounds was antagonistic to LVP in the rat pressor assay.

In the search for antagonists of the neurohypophyseal hormones, most work has been focused on oxytocins, and the antagonistic properties have been evaluated mainly in terms of oxytocin-like activities. The most potent inhibitors have proven to be analogues modified in position 1 alone or in combination with changes in positions 2 and 4 of the oxytocin molecule.[1,2]

Besides antagonizing the uterotonic action of oxytocin, some of these compounds also antagonize vasopressor responses to vasopressins. Thus, N-substituted [Tyr(Me)^2]oxytocins inhibit the vasopressor action of lysine-vasopressin (LVP) at inhibitor–LVP ratios[3] of about

0022-2623/78/1821-0352$01.00/0

1000–2000. The discovery[4] that some of these oxytocins retain their affinity for the kidney membrane receptor and act at the same time as antagonists in vitro recently encouraged Barth et al.[5] to investigate further their antagonistic properties in vivo. None of them appeared to antagonize either vasopressin or oxytocin in the antidiuretic assay.

Another series of modified oxytocins, namely, [Leu^4]-oxytocin,[6] [Phe^4]oxytocin,[7] and [Asp^4]oxytocin,[8] has been reported to inhibit the antidiuretic action of vasopressin. However, the antagonism became evident only at an enormous excess of the analogues, and, therefore, the antagonistic properties of these substances must be considered very weak.

In the vasopressin series, on the other hand, few analogues have been assayed for antagonistic properties. Arginine-vasopressinoic acid[9] exhibits an inhibition of adenylate cyclase in rat kidney in vitro, but to our knowledge its properties in vivo have not so far been investigated. Du Vigneaud and co-workers tried to apply their results from [Leu^4]oxytocin to vasopressin, but the analogue [Leu^4]-LVP[10] showed no inhibitory behavior in the antidiuretic test system. To further investigate the significance of positions 1 and 4 for inhibition, they also synthesized [(β-mercapto-β,β-diethylpropionic acid)^1]-LVP[11] and the corresponding 4-leucine analogue.[12] Both of them were oxytocin inhibitors but antagonized only the vasopressor effect of vasopressins. The 4-leucine series was completed with the synthesis of [Leu^4]arginine-vasotocin,[13] which showed weak agonistic activity in the antidiuretic and vasopressor assays.

Incorporation of *O*-methyltyrosine[14,15] and *O*-ethyltyrosine[15] into LVP yielded compounds with antagonistic properties similar to those observed for the corresponding oxytocins. Both behaved as agonists in the antidiuretic assay, the *O*-ethyl homologue, however, to a much lesser extent.

The significance of an O-alkylated tyrosine residue for agonism and antagonism primarily of oxytocin analogues has been discussed in general terms by Rudinger et al.[16] and more recently treated briefly by Barth et al.[17]

With this background we undertook the synthesis of LVP analogues, with and without a terminal amino group (i.e., with β-mercaptopropionic acid replacing Cys in position 1) and bearing different *O*-alkyl substituents in order to investigate their ability to act as anti-antidiuretic substances. The persisting interest in and the need for such analogues have recently been emphasized.[2,18]

Peptide Synthesis. *O*-*n*-Propyl-, *O*-*n*-butyl-, and *O*-*n*-hexyltyrosine were synthesized by reaction of the corresponding alkyl bromide with free tyrosine[19] and were then carbobenzoxylated under Schotten–Baumann conditions. Introduction of an ethyl group onto carbobenzoxytyrosine was done according to a procedure for carbobenzoxy-*O*-methyltyrosine[20] with the use of diethyl sulfate. The resulting *N*-carbobenzoxy-*O*-ethyltyrosine was isolated via its dicyclohexylammonium salt. Before incorporation into the partially protected heptapeptide, all tyrosine derivatives were converted to the corresponding *p*-nitrophenyl ester by standard procedures.[21]

The stability of the alkyl ethers under the conditions used for removal of the carbobenzoxy group was carefully investigated. The alkylated tyrosines were allowed to stand in 45% hydrogen bromide in acetic acid for 28 h (45 min was used for deprotection) and then run on an amino acid analyzer. No tyrosine could be detected in any case.

The protected nonapeptides were built-up stepwise in solution according to the *p*-nitrophenyl ester strategy.[22] Deprotection was performed using sodium in liquid ammonia, followed by oxidation in air (amino analogues) or with potassium ferricyanide. Purification was achieved by gel filtration and ion-exchange chromatography. The solubility and thus yields of the pure peptides decreased with increasing size of the alkyl substituent. The suprisingly low yield of the *O*-hexyl analogue could possibly have been increased by using mixtures of organic solvents and water for purification. Since this analogue was devoid of antagonistic properties, improvement of the synthesis was deemed superfluous.

Experimental Section

Amino acids were obtained from Ajinomoto Co., Inc., Tokyo, Japan, or from Tanabe Seiyaku Co., Osaka, Japan. All except for glycine were of the L configuration. TLC was performed on precoated silica gel 60 F_{254} plates (E. Merck AG, Darmstadt, Germany) in the following solvent systems [A, $CHCl_3$–MeOH–AcOH (85:10:5); B, *n*-BuOH–AcOH–H_2O (4:1:1); C, *n*-PrOH–H_2O (7:3); and D, *n*-BuOH–AcOH–pyridine–H_2O (15:3:10:6)] or on cellulose plates (Merck) [E, *n*-BuOH–AcOH–H_2O (4:1:5, upper phase); and F, *n*-BuOH–AcOH–pyridine–H_2O (15:3:10:12)]. TLE was done on precoated cellulose plates (Merck) in pyridine–AcOH–H_2O (11.5:3:485), pH 5.6, at 25 V/cm. Migration is expressed in centimeters in front of dinitrophenylethanolamine. Intermediate protected peptides and amino acids were detected by inspection under a UV lamp and by charring with $(NH_4)_2SO_4$ and free peptides by ninhydrin or o-phthalaldehyde.[23] For amino acid analysis peptides were hydrolyzed in sealed tubes for 24 h at 110 °C in 6 N HCl containing 1% (w/v) of phenol and analyzed on a Beckman Spinco analyzer. The macroreticular ion exchangers Amberlyst 15 (strong acid) and Amberlyst A-21 (weak base) were obtained from BDH Chemicals Ltd., Poole, England, and Sephadex from Pharmacia Fine Chemicals, Uppsala, Sweden.

The common protected heptapeptide was synthetized in one batch, using the same strategy and protecting groups as in ref 22. All analogues were prepared and purified by similar methods. Consequently, detailed experimental conditions will be given for one analogue only.

Properties of all intermediates are summarized in Table I.

Abbreviations used are Bzl, benzyl; DMF, dimethylformamide; LVP, lysine-vasopressin; Mpa, β-mercaptopropionic acid; ONp, *p*-nitrophenoxy; TLC, thin-layer chromatography; TLE, thin-layer electrophoresis; Tos, tosyl; and Z, carbobenzoxy.

Acid Stability of O-Alkylated Tyrosines. *O*-Ethyl-, *O*-*n*-propyl-, *O*-*n*-butyl-, and *O*-*n*-hexyltyrosine (10 μmol each) were dissolved in a mixture of 0.5 mL of AcOH and 0.5 mL of 45% HBr–AcOH. After stirring for 28 h aliquots of 0.5 mL were taken and evaporated. The residue was dissolved in 2.5 mL of pH 2.2 buffer, and 1 mL was applied to the short (7 cm) column of the amino acid analyzer. No tyrosine was detected in any run.

Z-Tyr(Et)-Phe-Gln-Asn-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2. Z-Phe-Gln-Asn-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2 (1.00 g, 0.86 mmol) was treated with HBr–AcOH for 45 min. The hydrobromide was precipitated with anhydrous ether, washed, dried, and dissolved in 7 mL of DMF. Triethylamine was added until the vapor was alkaline, followed by 0.48 g (1.03 mmol) of Z-Tyr(Et)-ONp. Coupling was allowed to proceed until TLC indicated the absence of ninhydrin-positive material (generally about 2 days). After complete reaction EtOH was added; and the precipitate was collected and washed with H_2O, EtOH, acetone, and EtOAc. Recrystallization from AcOH–EtOH yielded 0.93 g (79%) of the product.

Mpa(Bzl)-Tyr(Et)-Phe-Gln-Asn-Cys(Bzl)-Pro-Lys-(Tos)-Gly·NH_2. Deprotection and coupling were performed as described above except that *N*,*N*-diisopropylethylamine was used as the base in the coupling step. For final purification the product was dissolved in 2 mL of DMF and applied to a "mixed-bed ion-exchanger" (Amberlyst 15 + Amberlyst A-21, 1:1) column (1 × 8 cm) and eluted with 15 mL of DMF. The eluate was concentrated, and the material was precipitated with EtOH. After washing with EtOH and EtOAc, 0.67 g (75%) remained.

[Mpa^1,Tyr(Et)^2]-LVP. Protected nonapeptide (0.30 g) was deblocked with Na in liquid NH_3. The last traces of NH_3 were removed by lyophilization, and the residue was dissolved in 300

Table I. Properties of Intermediate Protected Amino Acids and Peptides

Compound	Yield (%)	Melting point[a] (°C)	Optical rotation in DMF $[\alpha]_D$ (deg)	R_f value[b] (TLC solvent system)	Analysis
Z-Tyr(Ethyl)	49	76- 78	-34.2[c] c1	0.64 (A) 0.68 (B)	-
Z-Tyr(Ethyl)-ONp	82	122-123	-12.9[d] c2	0.89 (A) 0.76 (B) 0.62 (C)	$C_{25}H_{24}N_2O_7$: C,H,N
Z-Tyr(Ethyl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2	79	213-216	-42.8[d] c0.5	0.50 (B) 0.63 (D)	Lys[e]; Cys(Bzl), 0.98; Asp, 1.03; Glu, 1.02; Pro, 1.01; Gly, 0.96; Tyr[f], 0.93; Phe, 0.99; NH_3, 3.2
Mpa(Bzl)-Tyr(Ethyl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2	75	219-221	-40.0[d] c0.5	0.50 (B) 0.66 (D)	-
Z-Tyr(n-Propyl)	71	87- 89	-31.3[d] c1	0.64 (A) 0.70 (B) 0.68 (D)	-
Z-Tyr(n-Propyl)-ONp	75	142-143	-12.8[d] c1	0.88 (A) 0.79 (B) 0.80 (D)	$C_{26}H_{26}N_2O_7$: C,H,N
Z-Tyr(n-Propyl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2	80	217-219	-41.7[d] c0.5	0.51 (B) 0.65 (D)	Lys[e]; Cys(Bzl), 0.98; Asp, 1.00; Glu, 1.04; Pro, 0.99; Gly, 1.00; Tyr[f], 0.78; Phe, 0.99; NH_3, 3.0
Mpa(Bzl)-Tyr(n-Propyl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2	77	222-225	-40.6[d] c0.5	0.50 (B) 0.66 (D)	-
Z-Tyr(n-Butyl)	82	91- 93	-33.6[c] c1	0.60 (A) 0.68 (B) 0.70 (D)	-
Z-Tyr(n-Butyl)-ONp	87	138-139	-12.5[c] c2	0.81 (B) 0.82 (D)	$C_{27}H_{28}N_2O_7$: C,H,N
Z-Tyr(n-Butyl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2	60	221-224	-39.8[d] c0.5	0.54 (B) 0.66 (D)	Lys[e]; Cys(Bzl), 0.95; Asp, 1.03; Glu, 1.05; Pro, 1.00; Gly, 1.00; Tyr[f], 0.80; Phe, 0.99; NH_3, 3.0
Mpa(Bzl)-Tyr(n-Butyl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2	83	222-224	-39.5[d] c0.5	0.55 (B) 0.68 (D)	-
Z-Cys(Bzl)-Tyr(n-Butyl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2	97	231-234	-43.2[d] c0.5	0.59 (B) 0.73 (D)	Lys[e]; Cys(Bzl), 1.99; Asp, 0.98; Glu, 1.03; Pro, 1.00; Gly, 1.00; Tyr[f], 0.81; Phe, 0.99; NH_3, 3.1
Z-Tyr(n-Hexyl)	67	80- 81	-31.1[c] c1	0.60 (A) 0.69 (B) 0.70 (D)	-
Z-Tyr(n-Hexyl)-ONp	81	129-130	-11.3[d] c2	0.90 (A) 0.80 (B)	$C_{29}H_{32}N_2O_7$: C,H,N
Z-Tyr(n-Hexyl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2	84	230-232	-44.2[d] c0.25	0.58 (B) 0.71 (D)	Lys[e]; Cys(Bzl), 0.96; Asp, 1.02; Glu, 1.00; Pro, 1.02; Gly, 1.01; Tyr[f], 0.76; Phe, 0.99; NH_3, 3.2
Mpa(Bzl)-Tyr(n-Hexyl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Lys(Tos)-Gly-NH_2	85	239-241	g	0.59 (B) 0.69 (D)	-

[a] Melting points were determined in open capillaries and are uncorrected. [b] Silica gel 60_{F254} plates (E. Merck AG). [c] 20°C. [d] 25°C. [e] Lysine was incompletely resolved, probably due to interference with Lys(Tos). [f] The O-alkyl substituents were not completely removed during hydrolysis. [g] This protected nonapeptide was not sufficiently soluble to allow optical rotation measurements.

mL of H_2O (deaerated and acidified with a few drops of AcOH). The reduced peptide was oxidized totally with 50 mL of 0.01 M $K_3Fe(CN)_6$ at pH 7.4. After stirring for 15 min excess oxidizing agent was removed with Dowex-2 (Cl^-), and the solution was lyophilized after adjustment of the pH to 3.5. The freeze-dried product was desalted in two portions on a column of Sephadex G-15 (1.4 × 140 cm) in 50% AcOH. Fractions of 3 mL were collected at a flow rate of 15 mL/h. The peptide eluted in fractions 29–35 as judged from the absorbance at 280 nm. After lyophilization of these fractions, the material was subjected to ion-exchange chromatography using a column of SP-Sephadex C-25 (3.1 × 6.5 cm). The sample was applied in 0.025 M ammonium formate, pH 3.6, and eluted with a 500-mL linear gradient (0.025–0.250 M ammonium formate, pH 3.6). The flow rate was 19.6 mL/h, and fractions of 5 mL were collected. The analogue was detected by UV absorbance at 280 nm and eluted in fractions 34–45. After repeated lyophilizations 86 mg of pure material remained. On TLE [Mpa[1],Tyr(Et)[2]]-LVP moved toward the cathode as a single spot, 2.9 cm in 45 min. TLC also indicated homogeneity (sample size 25–200 μg): R_f (E) 0.63 and R_f (F) 0.69. Amino acid analysis: Lys, 0.97; Cys (as cysteic acid), 1.01; Asp, 1.03; Glu, 1.02; Pro, 1.01; Gly, 0.98; Tyr, 0.93 (cf. Table I, footnote *f*); Phe, 1.00; NH_3, 3.2.

[Mpa[1],Tyr(*n*-Pr)[2]]-LVP. Protected nonapeptide (0.25 g) was deblocked and purified as described above. In the desalting step on Sephadex G-15 the peptide eluted in fractions 28–33. In the ion-exchange chromatography fractions 33–39 were recovered (5-mL fractions, flow rate 22 mL/h). The freeze-dried material was further purified by gel filtration on Sephadex G-15 in 0.2 M AcOH (column dimensions 1.4 × 114 cm). The peptide was applied in 3 mL of 0.2 M AcOH, the flow rate was 11 mL/h, and fractions of 3 mL were collected. Measurements of UV absorbance localized the product to fractions 41–54. The yield of lyophilized material was 25 mg. TLE showed one spot migrating 2.9 cm in 60 min. Homogeneity was further confirmed by TLC: R_f (E) 0.64 and R_f (F) 0.73. Amino acid analysis: Lys, 1.01; Cys, 1.02; Asp, 1.01; Glu, 1.01; Pro, 1.03; Gly, 0.98; Tyr, 0.78; Phe, 0.97; NH_3, 2.8.

[Mpa[1],Tyr(*n*-Bu)[2]]-LVP. Protected nonapeptide (0.25 g) was treated as above. From the ion exchanger the product eluted in fractions 34–42 and from the gel filtration column in tubes 52–64.

Table II. Influence of [Mpa¹,Tyr(alkyl)²]-LVP[a] on LVP-Induced Antidiuresis[b]

Analogue	Dose, pg/100-g body weight	No. of rats		Inhibition, % ± SE
		Total	Inhibited	
[Mpa¹,Tyr(Et)²]-LVP	2	4	0	
	2 × 10	8	6	74 ± 16
	2 × 10²	7	4	53 ± 12
	2 × 10³	5	1	47
	2 × 10⁴	3	0	
[Mpa¹,Tyr(*n*-Pr)²]-LVP	2	4	0	
	2 × 10	5	4	47 ± 18
	2 × 10²	6	2	28 ± 2
	2 × 10³	4	1	40
	2 × 10⁴	3	0	
[Mpa¹,Tyr(*n*-Bu)²]-LVP	2	4	0	
	2 × 10	14	8	31 ± 2
	2 × 10²	7	1	36
	2 × 10³	5	0	
	2 × 10⁴	3	0	
[Mpa¹,Tyr(*n*-hexyl)²]-LVP	2	4	0	
	2 × 10	4	0	
	2 × 10²	4	0	
	2 × 10³	3	0	

[a] LVP analogues were injected iv as single doses in 0.2 mL. [b] LVP was infused at a rate of 20 pg/100 g/min.

The yield of pure material was 11 mg. On TLE it moved 2.4 cm in 60 min, and on TLC the following R_f values were obtained: R_f (E) 0.65 and R_f (F) 0.77. Amino acid analysis: Lys, 0.98; Cys, 1.01; Asp, 1.03; Glu, 1.02; Pro, 1.04; Gly, 0.97; Tyr, 0.84; Phe, 0.97; NH_3, 3.0.

[Tyr(*n*-Bu)²]-LVP. Protected peptide (0.30 g) was deblocked and oxidized in air for 4 h at pH 7.4. After desalting on Sephadex G-15 in 50% AcOH, the product was applied to a column of SE-Sephadex C-25 in 0.025 M ammonium formate, pH 3.6. A 500-mL linear gradient was developed from 0.025 to 0.300 M ammonium formate, pH 3.6, for elution. The flow rate was 24 mL/h, and 5-mL fractions were collected. UV absorbance indicated the peptide in fractions 73–85. Finally, the peptide was applied to the same Sephadex G-15 column as the deamino analogues, and it eluted in fractions 43–55 at a flow rate of 14 mL/h. The yield of chromatographically pure material was 35 mg. On TLE it moved 3.2 cm in 60 min. TLC: R_f (E) 0.54 and R_f (F) 0.65. Amino acid analysis: Lys, 1.00; Cys, 2.00; Asp, 1.02; Glu, 1.01; Pro, 1.01; Gly, 0.99; Tyr, 0.89; Phe, 0.97; NH_3, 3.2.

[Mpa¹,Tyr(*n*-hexyl)²]-LVP. The deprotection of 0.30 g of protected nonapeptide and subsequent oxidation proceeded as expected. However, the lyophilisate only partly dissolved in the 50% AcOH used for desalting, and, furthermore, when applied to the ion-exchange column the analogue precipitated. A small portion could be extracted from the resin using *n*-BuOH–AcOH–H_2O (4:1:1). After two runs on a column of Sephadex LH-20 (2 × 95 cm) in *n*-BuOH–AcOH–H_2O (4:1:1), 3 mg of homogeneous material was obtained. It moved 2.0 cm in 60 min on TLE, and on TLC the R_f values were R_f (E) 0.68 and R_f (F) 0.83. Amino acid analysis: Lys, 0.96; Cys, 0.97; Asp, 1.03; Glu, 1.00; Pro, 1.00; Gly, 1.02; Tyr, 0.49; Phe, 0.96; NH_3, 3.0.

Antidiuretic tests were performed on anesthetized hydrated rats as follows. Female Sprague–Dawley rats weighing 180–220 g were anesthetized by intraperitoneal (ip) injection of 15 mg of sodium ethyl (1-methylpropyl)thiobarbiturate (Inactin) per 100-g body weight. A tracheotomy was performed, and one jugular vein was cannulated for intravenous (iv) administration of peptides in single doses or by permanent infusion. A polyethylene catheter was placed into the urethra for collection of urine. Diuresis was induced by permanent infusion of a hypoosmotic solution, containing 0.24% NaCl and 1.8% glucose, at a rate of 0.15 mL/min. Urine samples were collected at 5-min intervals, and the volume of each sample was measured.

Two methods were used for investigation of the antagonistic properties of the analogues.

(i) **Injection Experiments.** LVP and the analogue in question were administered together in single doses, and the response was compared with that to the same amount of LVP alone. The dose of LVP (50–100 pg per 100-g body weight) was chosen so as to reduce the urine excretion to about 50% of the original level.

(ii) **Infusion–Injection Experiments.** The constant diuresis induced by infusion of the hydration solution was reduced to about 50% by a permanent infusion of LVP at a rate of 20 pg/min/100 g. After a steady state in urine flow was reached, the analogues were administered iv as single doses in volumes of 0.2 mL. Injections of equal volumes of saline did not affect the steady-state diuresis.

Agonistic properties of the analogues were evaluated by means of the four-point dosage schedule.[24] Synthetic LVP (Ferring, AB, Malmö) was used as a standard.

Vasopressor activity was assayed on male Sprague–Dawley rats, body weight 250–325 g, under urethane anesthesia (10–15 mg/100 g ip). The right jugular vein was cannulated for administration of iv injections. In some experiments the femoral vein was cannulated for permanent infusion of LVP (10–20 ng/mL). A carotid artery was cannulated for recording blood pressure, using a Statham P23 DC transducer. Prior to injection of the first peptide, 6 × 1 mg of Ansolysen (Pharma Rodia) was given iv.

The following analogues were tested: [Tyr(Et)²]-LVP[15] (resynthesized at Ferring AB, Malmö), [Mpa¹,Tyr(Et)²]-LVP, [Mpa¹,Tyr(*n*-Pr)²]-LVP, [Tyr(*n*-Bu)²]-LVP, [Mpa¹,Tyr(*n*-Bu)²]-LVP, and [Mpa¹,Tyr(*n*-hexyl)²]-LVP. The dose of each analogue was chosen so as to cover a wide range of molar antagonist–agonist ratios.

Results

Antidiuresis. All of the LVP analogues tested showed agonistic activity with potencies in the range of 0.2–2.0 IU/μmol.

Inhibition of the LVP-induced antidiuretic effects was demonstrated when LVP was administered by a permanent infusion (cf. antidiuretic tests ii). Table II shows that the optimal dose for inhibition of the LVP-induced antidiuresis was 20 pg/100 g, when LVP was infused at a rate of 20 pg/100 g/min, and that there was a tendency for the inhibition to decrease with increasing length of the alkyl chain. In no instances were the inhibitory effects of the peptides protracted. Figure 1 shows the time course of one such infusion–injection experiment.

We were also able to demonstrate that in rats where the deamino analogues were antagonistic, no such effect was obtained with the corresponding amino analogues.

The antagonistic effect was not observed when LVP and the analogue were injected simultaneously (cf. antidiuretic tests i). On the other hand, at higher molar analogue–LVP ratios (250–1000) there was an enhancement of the antidiuretic response to LVP, presumably reflecting the

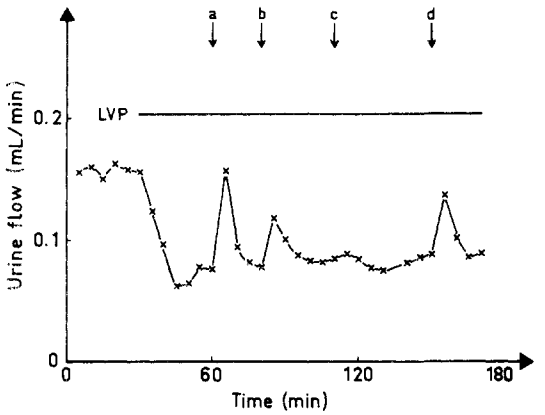

Figure 1. Inhibition by [Mpa[1],Tyr(Et)[2]]-LVP of the antidiuretic response to LVP in an infusion–injection experiment. Permanent infusion of LVP at a rate of 20 pg/min/100 g of body weight is indicated by the horizontal line. Single injections of the analogue are indicated by arrows: a, 20 pg; b, 200 pg; c, 2 ng; and d, again 20 pg, all per 100-g body weight.

agonistic activity of the analogue (additive effect).

Blood Pressure. Agonistic activity was shown by all tested analogues within about the same range as that observed for the antidiuresis, i.e., 0.5–3.0 IU/μmol. It was not possible to demonstrate antagonistic effects for any of the analogues, either in an infusion–injection or injection–injection experiment.

Discussion

The present results show that small doses of deaminated LVP analogues with certain alkyl substituents on the tyrosine residue can inhibit LVP-induced antidiuresis. The exact molar antagonist–agonist ratio which produces maximal inhibition for a given analogue is difficult to estimate since LVP and analogues were administered by different techniques. However, this ratio is certainly far below 100 (Table II). In some additional experiments where both antagonist and LVP were administered by infusion, a 100% inhibition was obtained at a molar ratio of 10. These preliminary experiments were performed with [Mpa[1],Tyr(*n*-Bu)[2]]-LVP. It is clear that our results differ significantly from earlier reports on antagonism of vasopressin in the antidiuretic assay,[6-8] where extremely high antagonist–agonist ratios were needed.

The *O*-methyl analogues were not included in our investigation and thus cannot be excluded as possible antidiuretic antagonists. However, this does not seem very likely since [Tyr(Me)[2]]-LVP is a much more potent agonist than [Tyr(Et)[2]]-LVP.[15]

Considering that only a small excess of the new analogues is needed to inhibit the antidiuretic response (Table II) and that these substances in high doses show agonistic properties, one is tempted to describe them as competitive inhibitors. Further support for this hypothesis is gained from the in vitro properties of similarly modified oxytocins,[4,25] the inhibitory action of which has been shown to be competitive in nature. However, explanation of the inhibition on a molecular level calls for more extensive investigations. Substitution of [Mpa[1]]-LVP for LVP as the agonist may help to elucidate whether the lack of inhibitory action in vivo of the amino analogues can be ascribed to differences in metabolic rate. Such experiments, in addition to studies of the in vitro properties of the new compounds, will be carried out in our laboratory in the near future. The present analogues were not inhibitory in the injection experiments nor were the inhibitory effects seen in all rats in the infusion–injection experiments. The reason for this also remains unknown and reflects the number of complex factors which affect the inhibitory action of peptide hormones.

Acknowledgment. This work was initiated together with Professor Josef Rudinger. Unfortunately, he did not live long enough to see any results. Thanks are due to Dr. Ulf Ragnarsson for many valuable discussions throughout this work and to Dr. Lars Carlsson for providing [Tyr-(Et)[2]]-LVP. Dr. James Halpert is gratefully acknowledged for linguistic revision of the manuscript. Financial support was given by The Swedish Natural Science Research Council (K 3020-002), Stiftelsen Uddeholms AB:s Stipendiefond (L.-E. L.), and by The Swiss National Science Foundation, Grant No. 3.040.76 (V.P.).

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