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Iodination of Vasopressin Analogues with Agonistic and Antagonistic Properties: Effects on Biological Properties and Affinity for Vascular and Renal Vasopressin Receptors

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SUMMARY

Twelve L- and D-tyrosine-containing vasopressin analogues were prepared in their mono- and diiodinated forms. These include six arginine vasopressin (AVP) vascular (V_1) type antagonists/antidiuretic (V_2) agonists, four V_1/V_2 antagonists, and two V_1/V_2 agonists, one of which is AVP itself. Ten peptides were iodinated on the tyrosyl residue in position 2; two were iodinated on a tyrosyl amide residue replacing the glycyl amide residue at position 9. All peptides were tested both for their biological activities *in vivo* (rat vasopressor and antidiuretic tests) and for their ability to bind to vasopressin receptors of the V_1 (vascular) and V_2 (renal) types from rat liver and rat kidney membranes, respectively. It is shown that monoiodination of the tyrosyl residue in the vasopressin analogues that were tested either preserves or reduces to a highly variable extent the *in vivo* and *in vitro* biological activities of these analogues. In most cases diiodonitation resulted in a marked decrease in biological activity. The effects of iodination on the affinity of vasopressin analogues for hepatic V_1 receptors and renal V_2 receptors were more related to the affinity of the noniodinated peptide for these receptors than to the biological properties (antagonist versus agonist) of the tested analogues, the nature (L versus D) of the iodinated tyrosyl residue, or the position (2 versus 9) at which this residue was introduced. The loss of affinity due to iodination was usually more pronounced for peptides exhibiting high affinity for vasopressin receptors. However, we show that among the monoiodinated peptides some (especially monoiodinated [2-D-Tyrosine]-AVP) retained enough affinity for vasopressin binding sites to suggest that their radioiodinated conterparts would be promising labeled ligands for use in studies in vasopressin receptors.

In mammals vasopressin exerts a large variety of biological actions. Although tritium-labeled vasopressin had allowed characterization of vasopressin receptors from different tissular sources, the lack of a radioactive ligand with both high specific activity and high receptor affinity has hampered many studies on vasopressin receptors. Two of us (1) recently showed that the same structural modifications of vasopressin analogues (deletion of the glycyl or glycineamide residue in position 9 and various amino acid substitutions in positions 2 and 4) have clearly different effects depending on the biological properties (agonistic versus antagonistic) of the parent molecule. The biological potency was usually better preserved in antagonists than in agonists. In line with this observation is a recent report by Moore *et al.* (2) indicating that iodination of a vasopressin antagonist preserved its antagonistic potency. It therefore appears that radioiodination of a chemically modified vasopressin molecule might provide a clue for the preparation of new ligands exhibiting high affinity for vasopressin receptors and high specific radioactivity. In the present study, we tested this working hypothesis by measuring the effects of iodination on the biological activities of a series of 12 vasopressin analogues. These included agonists and antagonists of the antidiuretic and vasopressor responses. We also investigated the effects of substitution of D- for L-tyrosine in position 2 and those of introducing a tyrosyl amide residue in place of glycine amide in position 9 of two vasopressin antagonists. For this purpose, [D-Tyr^2]-AVP (3) and two new vasopressin antagonists were synthetized and iodinated. All iodinated peptides were tested both for their biological activities *in vivo* and for their ability to bind to vasopressin receptors of the V_1 (vascular) and V_2 (renal) types (4).

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Materials and Methods

Preparation and characterization of iodinated vasopressin and vasopressin structural analogues. Table 1 lists the 12 L- and

ABBREVIATIONS: AVP, arginine vasopressin; LVP, lysine vasopressin; HPLC, high performance liquid chromatography; DMSO, dimethyl sulfoxide.

D-tyrosine-containing peptides that were used as precursors for the preparation of iodinated derivatives. These include six AVP vascular (V_1) type antagonists/antidiuretic (V_2) agonists (numbers 1–6), four V_1/V_2 antagonists (numbers 7–10), and two V_1/V_2 agonists, one of which is AVP itself (numbers 11 and 12). Ten of these peptides (numbers 1–5, 7–9, and 11 and 12) have been reported. Two new previously unpublished peptides were also used in this study. These are number 6, [1-(β-mercapto-β,β-cyclopentamethylene propionic acid), 2-D-tyrosine, 4-valine] arginine vasotocin (d(CH_2)$_5$-[D-Tyr^2]-VAVT) (M. Kruszynski, M. Manning, and W.H. Sawyer unpublished data) and number 10, [1-(β-mercapto-β,β-cyclopentamethylene propionic acid), 2-*O*-ethyl-D-tyrosine, 4-isoleucine, 9-D-tyrosinamide]-AVP (d(CH_2)$_5$[-D$Tyr(Et)^2$, Ile^4, $Tyr(NH_2)^9$]-AVP) (A. Misicka, M. Manning, and W.H. Sawyer, unpublished data). In addition, [2-D-tyrosine]-AVP ([D-Tyr^2]AVP) (3) was resynthetized (W.A. Kliss, M. Manning, and W.H. Sawyer, unpublished data) and used in this study. All syntheses were performed using the Merrifield solid phase method (5) following previously described procedures (6–10). Details of these syntheses will be reported elsewere.

[2-(3,5-diodotyrosine)] lysine vasopressin (diodo-LVP) was synthetized using the Merrified solid phase method (5). Boc-3,5-diodo-tyrosine (2,6-dichlorobenzyl) was directly incorporated in position 2 of the LVP sequence. Diiodo-LVP was purified by reverse-phase HPLC. The structure of the purified peptide was determined by [^{1}H]NMR spectroscopy. All spectra were recorded with a Brücker WM 360 WB spectrometer operating in the Fourier transform mode with quadrature detection at 360 MHz and equipped with an Aspect 3000 computer possessing an array processor. Two-dimensional homonuclear correlated (COSY) spectra were recorded using the pulse sequence (D_1-90°-D_2-45°-D_3)$_n$ (14), where D_2 and D_3 are the evolution and observation periods, respectively. Two-dimensional homonuclear dipolar correlated (NOESY) spectra in phase sensitive mode were acquired with pulse sequence (D_1-90°-D_2-90°-τ_m-90°-D_3)$_n$ (15) with a mixing time (τ_m) of 250 ms. COSY and NOESY experiments were acquired with 256 values of D_2 from 0.31 m to 79.87 ms.

The iodinated derivatives of the peptides in Table 1 were prepared as follows: 1 mg lyophilized peptide was dissolved in 1 ml ice-cold phosphate buffer (10 mM, pH 6.0). Three 10-μl aliqots of iodine chloride (70 mM) in methanol were added at 15-sec intervals under continuous stirring. The reaction was allowed to proceed at 0°C for 4 min and was stopped by immediate injection onto a HPLC (microbondapack C_{18} from Waters) column. The iodinated and nonreacted species were eluted with a linear gradient of trifluoroacetic acid, 0.1%, acetonitrile 75% (B) in triflouroacetic acid 0.1% (A). Depending on the overall hydrophobicity of the molecule, the gradient of B in A was adjusted to ensure optimal separation of the iodinated derivatives. The flow rate was 1 ml/min and the gradient built in 20 min. This procedure allowed a complete separation of the nonreacted, monoiodinated and diiodinated species that were eluted in hat order. The concentrations of the iodinated peptides in the eluates were measured by ultraviolet spectrometry using monoiodo and diiodotyrosine as standards. The purified products were concentrated under vacuum to eliminate organic solvents, suspended in water, and lyophilized.

[^{3}H]$_2$$Tyr^2$]-LVP ([^{3}H]vasopressin) was prepared as previously described (16) and purified by affinity chromatography using a neurophysin-sepharose column and by HPLC. The specific radioactivity of the labeled peptide was 17 Ci/mmol. Its biological activity was tested on the vasopressin-sensitive adenylate cyclase from LLCPK-1 cells (17) and was found identical to that of the starting material, synthetic lysine vasopressin from UCB-Bioproducts (Brussels, Belgium) repurified by HPLC.

Membrane preparations and [^{3}H]vasopressin binding assays. Animals used were Wistar rats (180–200 g body weight) purchased from IFFA CREDO (Lyon, France). Rat liver membranes were prepared following (up to step 11) the procedure described by Neville (18). They were stored in liquid nitrogen. Rat kidney membranes were prepared as described by Butlen *et al.* (19).

The binding assay on liver membranes was conducted as described in Ref. 20. Membranes (30 μg protein) were incubated for 30 min at 30°C in a total volume of 100 μl medium (Tris-HCl (pH 7.4) 50 mM, $MgCl_2$ 5 mM, bovine serum albumin 1 mg/ml [^{3}H]vasopressin, 5 nM) and increasing amounts of the unlabeled peptide to be tested. The binding assay on kidney membranes was conducted as described in Ref. 19). Membranes (100–200 μg protein) were incubated for 15 min at 30°C in 100 μl of a medium composed of Tris-HCl (pH 7.4) 50 mM, $MgCl_2$ 0.75 mM, EDTA-Tris 0.25 mM, bovine serum albumin 1 mg/ml,

TABLE 1

AVP and analogues: vasopressor (V_1) antagonists/antidiuretic (V_2) agonists (numbers 1–6), V_1/V_2 antagonists (numbers 7–10), and V_1/V_2 agonists (numbers 11 and 12) used for preparation of iodinated derivatives

No.	Peptide[a]	Biological activities		References
		Antivasopressor pA_2[a]	Antidiuretic activity U/μmol	
1	dVDAVP	7.03	1,295	6
2	dPDAVP	7.62	6.4	7, 8
3	d(Et_2)DAVP	7.96	0.075	8
4	d(Et_2)OVT	7.62	0.006	9
5	d(CH_2)$_5$DAVP	8.52	0.35	8
6	d(CH_2)$_5$[D-Tyr^2]VAVT	7.21	Mixed agonist/antagonist	•
7	d(CH_2)$_5$[D-Tyr^2]VAVP	8.41	Antagonist [pA_2 = 7.51]	10
8	d(CH_2)$_5$[D-Tyr^2]VDAVP	8.05	Antagonist [pA_2 = 7.03]	10
9	d(CH_2)$_5$[D-Phe^2,Ile^4,$Tyr(NH_2)^9$]AVP	7.27	Antagonist [pA_2 = 7.85]	11
10	d(CH_2)$_5$[D-$Tyr(Et)^2$,Ile^4,$Tyr(NH_2)^9$]AVP	8.10	Antagonist [pA_2 = 7.67]	•
11	AVP[d]	agonist 400 U/μmol	350	12
12	[D-Tyr^2]AVP[c]	agonist 94 U/μmol	463	•

[a] pA_2 is the negative logarithm of the molar concentration of antagonist that reduces the response to two times the units of agonist (AVP) to equal the response to one times the unit of agonist in the absence of antagonist (23). *In vivo* pA_2 values are estimated as the negative log of the effective dose (ED) divided by an assumed volume of distribution in the rat of 67 ml/kg (24). The ED is the dose of antagonist (in nmol/kg) that reduces the response to two times the units of agonist to equal the response to one times the unit of agonist administered in the absence of antagonist.

[b] dVDAVP, 1-deamino-[4-valine, 8-D-arginine]-vasopressin; dPDAVP, 1-deaminopenicillamine-[8-D-arginine]-vasopressin; d(Et_2)DAVP, [1-β-mercapto-β,β-diethylpropionic acid), 8-D-arginine]-vasopressin; d(Et_2)OVT, [1-(β-mercapto-β,β-diethylpropionic acid), 8-ornithine] vasotocin; d(CH_2)$_5$DAVP, [1-(β-mercapto-β-β-cyclopentamethylene propionic acid), 8-D-arginine]-vasopressin; d(CH_2)$_5$[D-Tyr^2]-VAVT, [1-(β-mercapto-β-β-cyclopentamethylene propionic acid), 2-D-tyrosine, 4-valine]-arginine vasotocin; d(CH_2)$_5$[D-Tyr^2]-VAVP, [1-(β-mercapto-β-β-cyclopentamethylene propionic acid), 2-D-tyrosine, 4-valine]-arginine vasopressin; d(CH_2)$_5$[D-Tyr^2]-VDAVP, [1-(β-mercapto-β-β-cyclopentamethylene propionic acid), 2-D-tyrosine, 4-valine, 8-D-arginine]-vasopressin; d(CH_2)$_5$[D-Phe^2,Ile^4,$Tyr(NH_2)^9$]-AVP, [1-(β-mercapto-β-β-cyclopentamethylene propionic acid), 2-D-phenylalanine, 4-isoleucine, 9-tyrosineamide]-arginine vasopressin; d(CH_2)$_5$[D-$Tyr(Et)^2$,Ile^4,$Tyr(NH_2)^9$]-AVP, [1-(β-mercapto-β-β-cyclopentamethylene propionic acid), 2-O-ethyl-D-tyrosine, 4-isoleucine, 9-tyrosineamide]-arginine vasopressin; [D-Tyr^2]-AVP, [2-D-tyrosine]-arginine vasopressin.

[c] This analogue was originally synthesized by Hruby *et al.* (3).

[d] Originally synthesized by du Vigneaud *et al.* (13).

• These analogues were synthesized by the Merrifield solid phase method (5), following previously described procedures (6–10).

[^{3}H]vasopressin 5nM) and increasing amounts of the unlabeled peptide to be tested. The reaction was initiated by the addition of membranes and stopped by adding 4 ml cold stopping solution (Tris-HCl (pH 7.4), 10 mM, $MgCl_2$ 1 mM) followed by immediate filtration through Millipore 0.45-μ filters and washing with 12 ml stopping solution. Nonspecific binding was determined in the presence of 10 μM unlabeled vasopressin. Radioactivity measurements were performed by liquid scintillation spectrometry. In each individual experiment dose-dependent inhibition of [^{3}H]vasopressin binding by unlabeled AVP and by five to six analogues was determined. Each peptide was tested at six different concentrations obtained by stepwise (1.63-fold) dilutions. The pK_d values for the unlabeled peptide were calculated as indicated in the legend to Fig. 2. All determinations were performed in duplicate, and the affinity of a given analogue was deduced from the results of two independent experiments.

***In vivo* bioassays of vasopressin agonists and antagonists.** All *in vivo* bioassays were done on female Sherman rats, 200–240 g, obtained from Camm Research Lab Animals, Wayne, NJ. Vasopressor assays were performed on rats anesthetized with urethane and treated with phenoxybenzamine (21). Antidiuretic assays were done by intravenous injection into hydrated rats under ethanol anesthesia (22). The U.S. Pharmacopoeia posterior pituitary reference standard was used in all assays. Assays for antagonistic potency were conducted on the same preparations by estimating "effective doses" of antagonists. The effective dose is defined as that dose that reduces the response to two times the units of agonist to equal the response to one times the units, that is, to half the dose (23). Doses of antagonist are found that are above and below the effective dose which is estimated by interpolation on a logarithmic scale. To obtain estimates of *in vivo* pA_2 values the effective dose is divided by an arbitrarily assumed volume of distribution (67 ml/kg) to obtain an estimated effective molar concentration of the antagonist (24). The negative logarithm of this concentration is the estimated *in vivo* pA_2. When mean pA_2 values are indicated with standard errors in the tables, these represent the means of at least four such determinations.

Results and Discussion

Iodination of vasopressin and vasopressin structural analogues. The [2-(3,5-diodotyrosine]-LVP prepared by direct synthesis was fully characterized by ^{1}H-NMR two-dimensional studies in DMSO-d_6. In a COSY experiment (14), the nine amino acids of the peptide sequence were identified and their positions determined by measuring the Overhauser effect between the NH_{i+1} and the $H\alpha_i$ protons assigned in a previous NOESY (15) experiment. The long-range coupling constants with $\beta\beta'$Tyr allowed us to assign the singlet at 7.64 ppm to the 2–6 aromatic protons of the 3,5-diiodotyrosyl residue. Finally, two observations indicated that the peptide was correctly cyclized. First, SH signals were not observed in the 2.4–2.2 ppm region. Second, the low temperature coefficient of the Asn^5 amide proton (−0.2 ppb/°C) indicated that it was involved in an intramolecular hydrogen bond (25) and that the four residues Tyr^2-Phe^3-Gln^4-Asn^5 formed a β turn structure. The more lipophilic product obtained after ICI treatment of LVP and the fully characterized [2-(3,5-diiodotyrosine)] LVP had identical retention times in reverse-phase HPLC when eluted in a gradient or isocratic mode. HPLC of a 1/1 mixture of the two products failed to reveal any heterogenity of the eluted peak. Both products exhibited the classical bathochrom shift of the ultraviolet spectrum due to dioiodination (λmax = 310 nm at basic pH). A less lipophilic product obtained after ICl treatment of LVP had the expected ultraviolet spectrum of a monoiodotyrosine-containing peptide (λmax = 304 nm). Increasing ICl/peptide concentration ratio in the reaction mixture reduced the relative proportion of this less lipophilic product formed and increased that of diiodo-LVP. Altogether the above observations indicated that the iodine chloride iodination method coupled to the HPLC purification procedure allowed rapid and convenient preparation of monoiodo-and diiodotyrosyl derivatives of vasopressin. Similar results were obtained with all vasopressin analogues used. In all cases, as exemplified by Fig. 1, only three peaks were eluted from the HPLC column which, on the basis of the ultraviolet absorbtion spectra, were identified to the nonreacted, monoiodinated, and diiodinated species. The iodination yield appeared almost completely independent of the nature and position of the tyrosyl residue in the molecule.

The results obtained with peptide 10 deserve special comments. First, preliminary experiments with an ethyltyrosine containing vasopressin analogue indicated that it could not be iodinated to any significant extent using iodine chloride as oxidizing agent. Second, no more than two reaction products could be evidenced. Third, in a situation were the ICl/peptide ratio was about two (see Materials and Methods), the percent

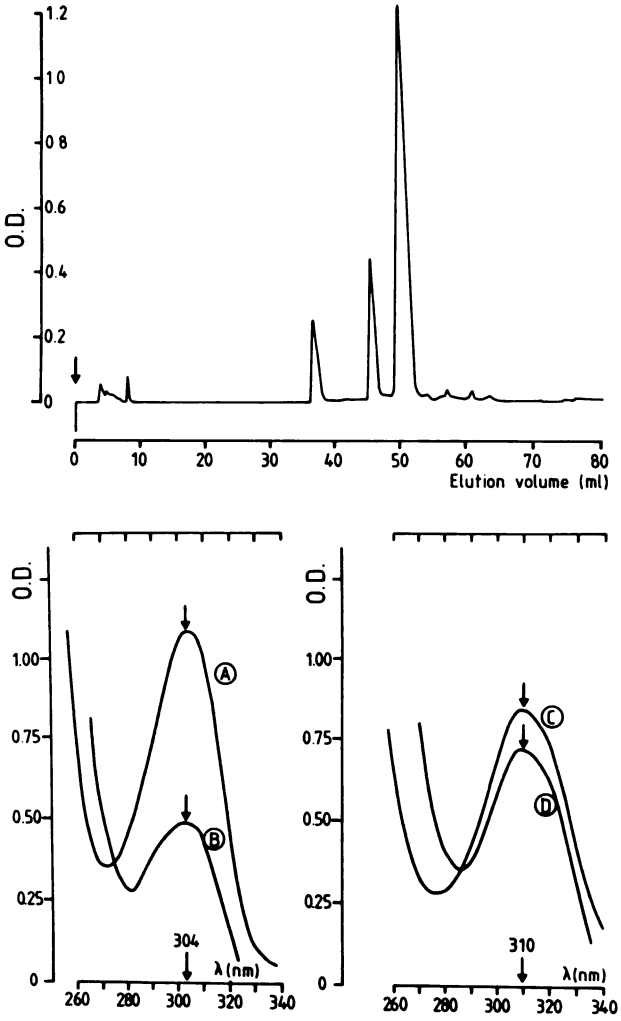

Fig. 1. Purification and spectrophotometric characterization of iodinated derivatives of vasopressin. AVP was iodinated as indicated under Materials and Methods. A, elution profile of HPLC column used for purification of vasopressin-ICl reaction products. The three sucessive peaks correspond to unreacted, monoiodotyrosine- and diiodotyrosine-vasopressins, respectively. Panel B, absorption spectra at basic pH of monoiodotyrosine-vasopressin (*B*), monoiodotyrosine (*A*), diiodotyrosine-vasopressin (*D*), and diiodotyrosine (*C*).

conversion of peptide 10 into more lipophilic products was similar to that observed with peptides containing only one tyrosyl residue. These observations indicate that the iodinated peptides that were prepared were the 9-mono- and 9-diodotyrosinamide derivatives of peptide 10.

***In vitro* binding assays.** Competition experiments on liver and kidney membranes (Fig. 2) revealed that all the unlabeled peptides tested inhibited [^{3}H]vasopressin binding to the same maximal extent, indicating that they interacted with the entire population of sites labeled with [^{3}H]vasopressin. The displacement curves were parallel. The slope indexes of these curves were close to one, suggesting the absence of microheterogeneity in the population of sites.

Effects of iodination on *in vitro* and *in vivo* vasopressor and antivasopressor properties. The data obtained on the rat liver membrane assay and the *in vivo* assays for vasopressor and antivasopressor activities are summarized in Tables 2 and 3. In line with the conclusions from previous studies by us and other groups (for review see Ref. 26), data obtained with antagonists of the vasopressor response (Table 2) indicated the existence of a significant correlation between the pK_d values determined on liver membranes and the corresponding *in vivo* pA_2 values. This holds for the parent analogues tested and their monoiodotyrosyl or diodotyrosyl derivatives. However, iodination probably introduced some general modifications in the antivasopressor potencies as they were determined *in vivo*. Indeed, the mean difference pA_2 − pK_d was 0.9 g for noniodinated peptides while the corresponding values for monoiodinated and diiodinated peptides were 0.72 and 0.2 g, respectively. Note that the pA_2 value is an operational and convenient way to estimate the antagonistic potency of a given compound but has no direct functional meaning in terms of affinity for the receptors involved. It is influenced by parameters such as volume of distribution, metabolic stability, half-life, and total plasma clearance. It is conceivable that iodination of vasopressin and analogues (with the resulting change in hydrophobicity of the molecule) could systematically affect one or several of these parameters. Anyway, the observed correlation between pK_d and pA_2 values confirms that hepatic and vascular vasopressin receptors have similar ligand specificities (26–27). It validates the choice of liver membranes as a model system with which to investigate the influence of iodination of vasopressin and vasopressin analogues on their affinity for vasopressin receptors of the V_{1a} (vascular) subtype (28).

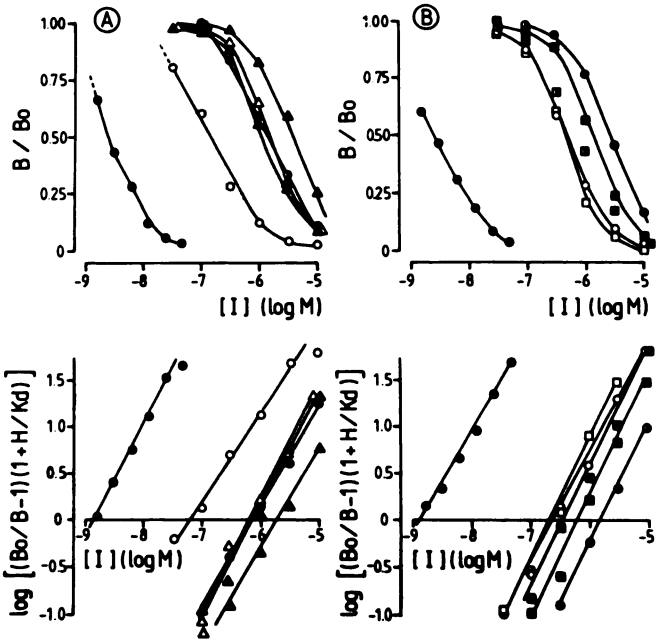

Fig. 2. Dose-dependent inhibition of [^{3}H]vasopressin binding by unlabeled vasopressin and vasopressin analogues. Specific [^{3}H]vasopressin binding to rat kidney membranes (*A*) and rat liver membranes (B) was measured in the presence of increasing amounts of the following unlabeled peptides: AVP (●), d(CH$_2$)$_5$-DAVP (▲), d(CH$_2$)$_5$-[D-Tyr^2]-VAVT (■), and their monoiodo (*open symbols*) and diiodo (*half-filled symbols*) derivatives. Values on graph are means of four determinations derived from two independent experiments. *Upper panels*, evolutions of B/Bo (*B*, specific binding in the presence of unlabeled peptide; Bo, specific binding in its absence) as a function of the concentration of unlabeled peptide (I) in the incubation medium. Note parallel displacement curves. *Lower panels*, log ((Bo/B)-1)(1+(H*/K_d)) is plotted as a function of log[I]. Lines on graphs were computed by linear regression analysis. H*, concentration of [^{3}H]vasopressin in the incubation medium; K_d, equilibrium dissociation constant for [^{3}H]vasopressin binding. The latter value was determined independently for each series of experiments. The pK_d values (pK_d = -log K_d) for the unlabeled peptides were deduced from the *x* intercept of the computed regression lines. The precision of these determinations was calculated as $S_x \times (1 - r^2)^{0.5}$ where S_x is standard error on *x* values and *r* is correlation coefficient.

For eight of eleven antagonists of the vasopressor response, introduction of one iodine atom led to a slight (up to threefold) increase in affinity. No clear differential effect of iodination could be observed between L-tyrosine and D-tyrosine containing peptides. With the exeption of peptides 6, 9, and 10, introduction of two iodine atoms resulted in a 2- to 30-fold loss in affinity. The new analogues with a tyrosylamide in place of a glycylamide residue in position 9 deserve special comments. This modification did not markedly affect their antivasopressor potency *in vivo*; d(CH$_2$)$_5$[D-Phe^2, Ile^4, Tyr(NH$_2$)^9]-AVP has a pA_2 value of 7.27 and its glycyneamide^9-containing counterpart a pA_2 of 7.86 + 0.05 (29). d(CH$_2$)$_5$ [D-Tyr(Et)^2, Ile^4]-AVP and *d*(CH$_2$)$_5$ [D-Tyr(Et)^2, Ile^4, Tyr(NH$_2$)^9]-AVP have very similar pA_2 values (7.78 and 8.10, respectively). This observation is a further illustration of the fact that antagonists are less sensitive than agonists to structural modifications of the C-terminal three amino acid side chain (1). Iodination of the Tyr(NH$_2$)^9 residue resulted in a slight increase in receptor affinity, whereas the PA_2 values were significantly decreased.

Iodination of the tyrosyl residue in the AVP molecule resulted in a marked loss in affinity for liver membranes (170 and 1,000-fold reduction for the monoiodo and dioiodo derivatives, respectively). Introduction of a D-tyrosyl residue in position 2 of the vasopressin molecule preserved a high vasopressor activity (Table 3). Iodination of this residue conferred antagonistic properties to the molecule (Table 2) and was accompanied by a marked reduction in affinity (30-fold in the case of the diiodinated derivative).

Effects of iodination in *in vitro* and *in vivo* antidiuretic and antiantidiuretic properties. The results obtained on the rat kidney membrane binding assay and the *in vivo* assays for antidiuretic and antiantidiuretic potencies of the analogues tested are summarized in Tables 4 and 5. Iodination of the tyrosyl and tyrosylamide residues in positions 2 and 9 of the vasopressin antagonists tested preserved their antagonistic properties. For the entire series of vasopressin antagonists including the monoiodo and diiodo derivatives, there was a significant correlation between pK_d values deter-

TABLE 2

Vasopressin analogues with antivasopressor activity

The affinity of the tested analogues (for abbreviations see Table 1) and their iodinated derivatives for liver membranes was determined as indicated in Materials and Methods and the legend to Fig. 2. Results are expressed in terms of pK_d ($pK_d = -\log K_d$). The correlation coefficient between pK_d and pA_2 values was 0.782 ($p < 0.01$). The mean differences $pA_2 - pK_d$ were 0.99, 0.72, and 0.29 for the noniodinated, monoiodinated, and diiodinated peptides, respectively.

Peptide tested	pK_d	Change in pK_d due to iodination	*In vivo* activities (antivasopressor test)	
			pA_2	Change in pA_2 due to iodination
dVDAVP (1)	6.03 ± 0.09		7.03 ± 0.11	
Monoiodotyrosine derivative	6.25 ± 0.08	+0.22	6.85 ± 0.07	−0.18
Diiodotyrosine derivative	5.57 ± 0.13	−0.46	<5.8	>−1.23
dPDAVP (2)	6.71 ± 0.05		7.62 ± 0.06	
Monoiodotyrosine derivative	7.28 ± 0.10	+0.57	7.93 ± 0.08	+0.31
Diiodotyrosine derivative	6.39 ± 0.07	−0.32	6.36 ± 0.05	−1.26
d(Et_2)DAVP (3)	6.89 ± 0.08		7.96 ± 0.08	
Monoiodotyrosine derivative	7.11 ± 0.11	+0.22	8.21 ± 0.04	+0.25
Diiodotyrosine derivative	6.33 ± 0.10	−0.56	6.50 ± 0.04	−1.46
d(Et_2)-OVT (4)	7.00 ± 0.08		7.62 ± 0.06	
Monoiodotyrosine derivative	7.03 ± 0.08	+0.03	8.05 ± 0.07	+0.43
Diiodotyrosine derivative	5.67 ± 0.10	−1.33	<5.75	>1.87
d(CH_2)$_5$-DAVP (5)	7.35 ± 0.14		8.52 ± 0.03	
Monoiodotyrosine derivative	7.36 ± 0.01	+0.01	8.30 ± 0.03	−0.22
Diiodotyrosine derivative	6.64 ± 0.13	−0.71	6.80	−1.72
d(CH_2)$_5$-[D-Tyr^2]-VAVT (6)	6.27 ± 0.24		7.21 ± 0.21	
Monoiodotyrosine derivative	6.68 ± 0.03	+0.41	7.17 ± 0.1	−0.04
Diiodotyrosine derivative	6.35 ± 0.14	+0.08	6.83 ± 0.03	−0.38
d(CH_2)$_5$-[D-Tyr^2]-VAVP (7)	7.17 ± 0.15		8.41 ± 0.11	
Monoiodotyrosine derivative	7.07 ± 0.13	−0.10	7.60 ± 0.06	−0.81
Diiodotyrosine derivative	6.82 ± 0.24	−0.35	7.57 ± 0.11	−0.84
d(CH_2)$_5$-[D-Tyr^2]-VDAVP (8)	7.43 ± 0.07		8.05 ± 0.03	
Monoiodotyrosine derivative	7.05 ± 0.10	−0.38	7.45 ± 0.17	−0.60
Diiodotyrosine derivative	6.91 ± 0.11	−0.52	7.48 ± 0.09	−0.57
d(CH_2)$_5$-[D-Phe^2-Ile^4-$Tyr(NH_2)^9$]-AVP (9)	6.64 ± 0.08		7.27 ± 0.05	
Monoiodotyrosine derivative	6.86 ± 0.13	+0.22	6.88 ± 0.12	−0.39
Diiodotyrosine derivative	6.80 ± 0.09	+0.16	6.65 ± 0.09	−0.62
d(CH_2)$_5$-[D-$Tyr(Et_2)^2$-Ile^4-$Tyr(NH_2)$]-AVP (10)	6.79 ± 0.09		8.10 ± 0.01	
Monoiodotyrosine derivative	6.95 ± 0.04	+0.16	7.63 ± 0.01	−0.47
Diiodotyrosine derivative	7.13 ± 0.30	+0.34	7.20 ± 0.07	−0.90
[D-Tyr^2]-AVP (11)	8.25 ± 0.20		agonist	
Monoiodotyrosine derivative	7.78 ± 0.04	−0.47	7.38 ± 0.07	
Diiodotyrosine derivative	6.72 ± 0.11	−1.53	6.70 ± 0.03	

TABLE 3

Vasopressin analogues with vasopressor activity

For abbreviations and details see Table 1. To compare *in vivo* and *in vitro* results, vasopressor activities and affinities for rat liver membranes were expressed with reference to AVP used as a standard. (pK_d analogue − pK_d AVP) = log (K_d AVP/K_d analogue): log relative activity = log (activity of analogue/activity of AVP).

Peptide tested	pK_d	Affinity for rat liver membranes		*In vivo* activities (*in vivo* vasopressor assay)		
		Relative affinity (AVP standard)	Change in affinity due to iodination	Vasopressor activity	Relative activity (AVP standard)	Change in activity due to iodination
		log		*units/μmol*	*log*	
AVP (11)	8.88 ± 0.03	0.00		400 ± 7	0.00	
Monoiodotyrosine derivative	6.65 ± 0.05	−2.23	−2.23	0.76 ± 0.03	−2.72	−2.72
Diiodotyrosine derivative	5.86 ± 0.01	−3.02	−3.02	0.60 ± 0.02	−2.82	−2.82
[D-Tyr^2]-AVP (12)	8.25 ± 0.20	−0.63		94 ± 5	−0.53	
Monoiodotyrosine derivative	7.78 ± 0.04	−1.10	−0.47	antagonist		
Diiodotyrosine derivative	6.72 ± 0.11	−2.16	−1.53	antagonist		

mined on rat kidney membranes and the corresponding pA_2 values *in vivo*.

Iodination of antagonists of the antidiuretic response resulted in either an increase (monoiodo derivative of peptide 6) or a decrease (up to 10-fold in the case of the diiodo derivative of peptide 10) in affinity for rat kidney membranes. Considering the vasopressin analogues with high antidiuretic potency (AVP, [D-Tyr^2]-AVP, and dVDAVP), iodination resulted in a variable loss in affinity (from 14-fold for monoiodo- [D-Tyr^2]-AVP to 3,300-fold in the case of diiodo-AVP). For analogues with low antidiuretic activities, the relative reduction in affinity due to iodination was less pronounced.

In summary, our results confirm the short report by Moore *et al.* (2) that monoiodination of the tyrosyl residue in a vasopressin antagonist, d(CH_2)$_5$-[D-Tyr^2, Val^4]-AVP, preserved a fairly high affinity for binding to hog kidney V_2 receptors.

TABLE 4

Vasopressin analogues with antiantidiuretic activities

For abbreviations see Table 1. For details, see Table 2. The correlation coefficient between pK_d and pA_2 values was 0.63 ($0.02 < p < 0.05$).

Peptide tested	Affinity for rat kidney membranes		In vivo activities (antiantidiuretic test)	
	pK_d	Change in pK_d due to iodination	pA_2	Change in pA_2 due to iodination
d(CH$_2$)$_5$-[D-Tyr^2]-VAVT (6)	6.54 ± 0.11		mixed agonist and antagonist	
Monoiodotyrosine derivative	6.80 ± 0.06	±0.26		
Diiodotyrosine derivative	6.36 ± 0.13	−0.18		
d(CH$_2$)$_5$-[D-Tyr^2]-VAVP (7)	7.22 ± 0.21		7.51 ± 0.08	
Monoiodotyrosine derivative	6.86 ± 0.15	−0.36	7.21 ± 0.10	−0.30
Diiodotyrosine derivative	6.81 ± 0.08	−0.41	6.51 ± 0.08	−1.00
d(CH$_2$)$_5$-[D-Tyr^2]-VDAVP (8)	7.38 ± 0.17		7.03 ± 0.05	
Monoiodotyrosine derivative	7.17 ± 0.16	−0.21	6.20 ± 0.03	−0.83
Diiodotyrosine derivative	6.84 ± 0.21	−0.54	6.07 ± 0.96	
d(CH$_2$)$_5$-[D-Phe^2-Ile^4-Tyr(NH$_2$)^9]-AVP (9)	7.43 ± 0.24		7.85 ± 0.06	
Monoiodotyrosine derivative	7.04 ± 0.08	−0.39	7.55 ± 0.08	−0.30
Diiodotyrosine derivative	7.22 ± 0.10	−0.21	7.23 ± 0.05	−0.62
d(CH$_2$)$_5$-[D-Tyr(Et)^2-Ile^4-Tyr(NH$_2$)^9]-AVP (10)	7.61 ± 0.46		7.67 ± 0.08	
Monoiodotyrosine derivative	7.23 ± 0.12	−0.38	7.60 ± 0.07	±0.07
Diiodotyrosine derivative	6.56 ± 0.13	−1.05	7.67 ± 0.11	0.00

TABLE 5

Vasopressin analogues with antidiuretic activity

For abbreviations see Table 1. For details, see Tables 2 and 3. The correlation coefficients between relative antidiuretic activities and relative affinities for rat kidney membranes were 0.981 and 0.784 ($p < 0.01$) for the noniodinated and monoiodinated peptides, respectively. For diiodinated peptides the range of variation of individual values was too narrow to allow a meaningful correlation analysis.

Peptide tested	Affinity for rat kidney membranes			*In vivo* activities (antidiuretic test)			
	pK_d	Relative affinity (AVP standard)	Change in affinity due to iodination	Antidiuretic activity		Relative activity (AVP standard)	Change in activity due to iodination
		log		*units/μmol*		*log*	
AVP (12)	8.89 ± 0.06	0.00		350	± 17	0.00	
Monoiodotyrosine derivative	6.14 ± 0.34	−2.75	−2.75	2.0	± 0.1	−2.24	−2.24
Diiodotyrosine derivative	5.37 ± 0.05	−3.52	−3.52	1.0	± 0.2	−2.54	−2.54
[D-Tyr^2]-AVP (11)	8.57 ± 0.29	−0.32		463	± 49	+0.12	
Monoiodotyrosine derivative	7.43 ± 0.08	−1.46	−1.14	38	± 2	−0.96	−1.09
Diiodotyrosine derivative	6.76 ± 0.06	−2.13	−1.81	0.95	± 0.1	−2.56	−2.66
dVDAVP (1)	9.06 ± 0.23	+0.17		1295	± 179	+0.57	
Monoiodotyrosine derivative	7.08 ± 0.04	−1.81	−1.98	1.1	± 0.1	−2.50	−3.0
Diiodotyrosine derivative	6.00 ± 0.30	−2.89	−3.06	0.5	± 0.1	−2.85	−3.41
dPDAVP (2)	7.16 ± 0.06	−1.73		6.4	± 0.8	−1.74	
Monoiodotyrosine derivative	6.30 ± 0.22	−2.59	−0.86	0.013	± 0.001	−4.43	−2.69
Diiodotyrosine derivative	6.62 ± 0.15	−2.27	−0.54	0.04	± 0.01	−3.94	−2.20
d(Et$_2$)-DAVP (3)	5.96 ± 0.08	−2.93		0.075	± 0.005	−3.67	
Monoiodotyrosine derivative	6.03 ± 0.18	−2.86	+0.07	0.002		−5.24	−1.57
Diiodotyrosine derivative	5.74 ± 0.03	−3.15	−0.22	0.0004		−5.94	−2.27
d(CH$_2$)$_5$-DAVP (5)	5.70 ± 0.17	−3.19		0.35		−3.00	
Monoiodotyrosine derivative	6.15 ± 0.17	−2.74	+0.45	0.002		−5.24	−2.24
Diiodotyrosine derivative	5.69 ± 0.09	−3.20	−0.01	0.005		−4.85	−1.84

Indeed, the K_d values for binding of this antagonist and its monoiodinated derivative to rat kidney membranes (60 and 138 nM, respectively, see Table 4) are close to the corresponding values of 82 and 118 nM reported by Moore *et al.* (2) for hog kidney membranes. We extend this conclusion in the case of V_1 receptors and show that the *in vivo* antivasopressor and antiantidiuretic properties of this and other vasopressin antagonists are preserved by iodination. However, the potency of these antagonists was in most cases altered by iodination.

The systematic study that was performed provides some insight on the effects of iodination on the affinity of vasopressin analogues for hepatic V_{1a} receptors and renal V_2 receptors. From the results shown in Tables 2–5 it appears that 1) the reduction in affinity due to monoiodination (when observed) was more pronounced for agonists (Table 3 and 5) than for antagonists (Table 2 and 4), 2) L- or D-tyrosine substitution at position 2 (compare peptides 1–5 and 11 to peptides 6–8 and 2 in Tables 2–5) or introduction of a tyrosinamide in place of glycine amide at position 9 (peptides 9–10) has no marked influence on the effects of iodination.

Altogether our results suggest, as illustrated by Fig. 3, that the effects of iodination on the affinity of vasopressin analogues for hepatic V_{1a} receptors and renal V_2 receptors are more related to the affinity of the noniodinated peptide for these receptors than to their biological properties (antagonist versus agonist) or structural features. The higher the affinity of the parent molecule, the more pronounced was the loss in affinity due to iodination. This might simply indicate that in situations where

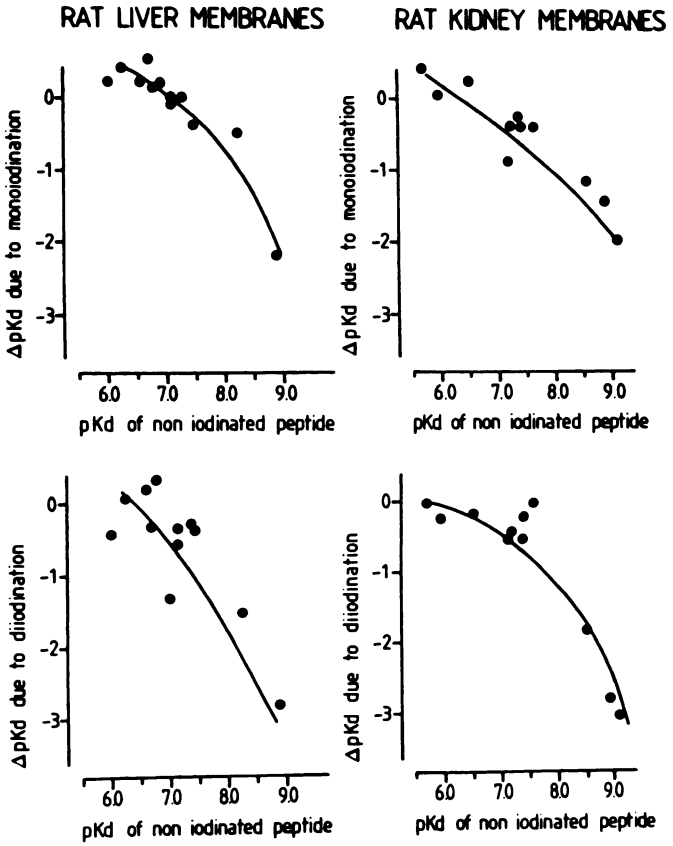

Fig. 3. Changes in affinity due to iodination of vasopressin agonists and antagonists: relation to the affinity of the noniodinated counterpart. Data also presented in Tables 2–5. Each dot corresponds to a vasopressin structural analogue that were tested. The change in affinity due to monoiodination (*upper panels*) and diiodination (*lower panels*) was measured as the difference between the pK_d value of the iodinated species and the pK_d value of the corresponding noniodinated peptide. These differences are plotted as a function of the corresponding pK_d values of the noniodinated peptides. Note that, for both rat liver (*left panels*) and rat kidney (*right panels*) membranes, the higher the affinity of the noniodinated peptide, the larger the reduction in affinity due to iodination of the tyrosyl residue in the molecule.

a close conformational complementarity between the receptor and the ligand exists (agonists and antagonists of high affinity), introduction of one or two iodine atoms will markedly affect the ligand-receptor interaction. However, we show that among the monoiodinated peptides that have been prepared some retained enough affinity for vasopressin binding sites to suggest that their radioiodinated counterparts would be promising labeled ligands for use in studies on vasopressin receptors. Thus, the pK_d of monoiodinated [D-Tyr^2]-AVP for V_1 receptors on hepatocytes is 7.78, indicating an affinity only about 13 times less than that of AVP (pK_d = 8.88). The potential gain in specific radioactivity, the relative ease and economy of preparation, and the substantial gain in counting efficiency could make radioiodinated [D-Tyr^2]-AVP a more efficient labeled ligand than [^{3}H]-AVP for studies on vasopressin receptors. Note that many of these studies, such as autoradiographic localization of vasopressin receptors in the brain and other tissues, for instance, are presently hampered by lack of a radioactive ligand with high specific activity.

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