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Vasopressin Antagonists Allow Demonstration of a Novel Type of Vasopressin Receptor in the Rat Adenohypophysis

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SUMMARY

The ligand specificity of rat adenohypophyseal vasopressin receptors was directly compared to that of peripheral receptors of the V_1 and V_2 types. For this purpose a series of 15 recently designed vasopressin antagonists was used. The affinities of these antagonists for rat adenohypophyseal membranes were deduced from the determination of the concentration-dependent inhibition of [^{3}H]vasopressin binding. In parallel experiments the corticotropin (or anti-corticotropin)-releasing activities of the tested peptides were determined on freshly dispersed rat adenohypophyseal cells. All peptides tested which were found to be antagonists of the vasopressor and antidiuretic responses to vasopressin *in vivo* behaved as antagonists of vasopressin-induced corticotropin release. There was a close correlation between the relative affinities of the analogues tested for binding to adenohypophyseal membranes and their relative potencies in inhibiting vasopressin-induced corticotropin release, indicating that the detected vasopressin-binding sites are the receptors involved in the vasopressin effect on corticotropin secretion. No correlation could be demonstrated between anti-corticotropin-releasing activities and either anti-antidiuretic or antivasopressor potencies of the antagonists tested. A direct comparison of the ligand specificities of adenohypophyseal receptors on the one hand, and V_1 (hepatic) and V_2 (renal) receptors on the other hand, showed that most of the antagonists discriminated very efficiently between adenohypophyseal and either hepatic or renal receptors. The selectivity index reaches values as high as 260,000 for desGly(NH$_2$)^9 [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid), 2-D-O-ethyl-tyrosine, 4-valine] arginine vasopressin. It is concluded that adenohypophyseal receptors represent a novel type of vasopressin receptors. Based on the observation that adenohypophyseal receptors, like hepatic or vascular V_1 receptors, do not appear to be coupled to adenylate cyclase, we propose that adenohypophyseal receptors could be designated as V_{1b} receptors as opposed to the V_{1a} receptors previously characterized on liver and blood vessels.

The multimolecular nature of the hypothalamic factors which control corticotropin release from the adenohypophysis is now well recognized (1–3). A number of recent experimental data clearly suggest that vasopressin might be one physiologically relevant component of this multifactorial regulation of corticotropin release in rats as well as in humans (4–8).

Two types of peripheral vasopressin receptors have been distinguished on both functional and pharmacological bases (9). V_2 receptors are functionally coupled to adenylate cyclase and have the same ligand specificity as the receptors involved in the antidiuretic response to vasopressin. V_1 receptors have the same ligand specificity as the receptors involved in the vasopressor or glycogenolytic responses to vasopressin and are not coupled to adenylate cyclase. They mediate the activation of calcium-dependent regulatory pathways (10). The nature of the adenohypophyseal vasopressin receptors is still controversial with respect to the two criteria mentioned above. Vasopressin was shown to potentiate corticotropin-releasing factor-induced cyclic AMP accumulation in isolated hypophyseal cells (11) and adenohypophyseal tissue incubated *in vitro* (12). However, in a previous study (13), we found that vasopressin does not affect the adenylate cyclase activity of rat adenohypophyseal membranes when investigated in experimental conditions where activation by corticotropin-releasing factor and inhibition by angiotensin II could be easily demonstrated. It was tentatively concluded that adenohypophyseal receptors are not coupled to adenylate cyclase and might therefore by of the V_1 type. A possible primary involvement of calcium in the corticotropin-releasing effect of vasopressin has not been directly demonstrated. However, it can be considered as likely in view

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ABBREVIATIONS: [^{3}H]vasopressin, [^{3}H$_2$Tyr^2]-lysine vasopressin; EBSS, Earle's balanced salt solution; EDTA, ethylenediaminetetraacetic acid. See Table 1, Footnote *b* for additional abbreviations.

of the recent report that vasopressin stimulates phosphatidylinositol-4,5-bisphosphate hydrolysis by rat adenohypophysis (14) and adenohypophyseal cells in primary cultures.[1] Although *in vivo* corticotropin-releasing activity of vasopressin analogues seems to parallel pressor potency (15), it was recently proposed by several groups that the ligand specificity of adenohypophyseal receptors might be different from those of receptors involved in the pressor and antidiuretic effects of vasopressin (16–19). Of special interest is the observation that potent antagonists of the vasopressor response to vasopressin, deamino (1-penicillamine, 2-*O*-methyltyrosine) arginine vasopressin, and [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid), 2-*O*-methyltyrosine] arginine vasopressin were poorly effective in blocking the corticotropin-releasing activity of vasopressin (17, 19). In the present study, we explored the possibility that vasopressin antagonists might provide the clue for the identification of some specific features of pituitary vasopressin receptors in terms of structure-activity relationships. Several groups (17, 20, 21) including ours (13) recently characterized specific vasopressin-binding sites on rat adenohypophyseal membranes and provided evidence that these sites are very likely the receptors involved in the corticotropin-releasing action of vasopressin. This receptor assay combined with the *in vitro* assay of corticotropin release by isolated adenohypophyseal cells allows a direct examination of the ligand specificity of adenohypophyseal receptors. Using a series of recently designed vasopressin antagonists (22–24), we demonstrate that adenohypophyseal vasopressin receptors do in fact represent a novel type of receptor exhibiting a ligand specificity strikingly different from those of V_2 (antidiuretic) and V_1 (vasopressor or glycogenolytic) receptors.

Materials and Methods

Chemicals. Arginine vasopressin analogues used are listed in table 1. The legend to table 1 includes references for the synthesis and pharmacological properties of these analogues. [$^3H_2$Tyr^2]-lysine vasopressin ([^{3}H]vasopressin) was prepared as previously described (25) and purified by affinity chromatography using a neurophysin-Sepharose column followed by high pressure liquid chromatography. The specific radioactivity of the labeled peptide was 17 Ci/mmol. Its biological activity was tested on the vasopressin-sensitive adenylate cyclase from LLC-PK$_1$ cells (26). It was found to be identical to that of the starting material, synthetic lysine-vasopressin from UCB-Bioproducts (Brussels, Belgium), repurified by high pressure liquid chromatography. Trypsin and lima bean trypsin inhibitor were purchased from Worthington Biochemical Corp. (Freehold, NJ); Earle's balanced salt solution (EBSS), from GIBCO (Grand Island, NY); Trasylol, from Bayer (Haywards Heath, Sussex, England); and Biogel P-2 (200–400 mesh) from Bio-Rad Laboratories (Richmond, CA). All other chemicals were of A grade purity.

Preparations. Animals used were female Wistar rats (180–200 g of body weight) purchased from IFFA CREDO (Lyon, France). For each [^{3}H]vasopressin binding experiment, 50–85 adenohypophyses were collected and gently homogenized in 20 mM $NaHCO_3$ by using a Dounce homogenizer. The homogenate was stirred at 4° for 15 min and spun at 200 × *g* for 20 min. The supernatant was filtered twice through nylon gauze (20 μm) and then centrifuged at 30,000 × *g* for 30 min. The pellet was resuspended in cold binding assay buffer and used immediately.

Rat liver membranes were prepared following (up to step 11) the procedure described by Neville (27). They were stored in liquid nitrogen. Rat kidney membranes were prepared as described by Butlen *et al.* (28).

The isolated rat anterior pituitary cell column used for corticotropin-releasing factor bioassay was prepared as described by Gillies and Lowry (29). Briefly, the cells from five adenohypophyses were dispersed by mechanical agitation in 0.25% trypsin solution, mixed with 0.5 g of preswollen Biogel P-2 (200–400 mesh), and packed into a 2-ml plastic column (0.9 × 3 cm). Cells were counted with a hemocytometer and stained with trypan blue (0.2%) for the estimation of viability. Cell viability exceeded 90% and remained at this high level for more than 10 hr. The column was washed with EBSS containing 0.05% lima bean trypsin inhibitor and subsequently was perfused at a rate of 0.5 ml/min with EBSS containing ascorbic acid (50 μg/ml), 0.25% human serum albumin, Trasylol (100 kallikrein inactivation units/ml), and antibiotics (15 μg of benzyl penicillin and 25 μg of streptomycin per ml). The column and the perfusion medium were maintained at a temperature of 37°. The perfusion medium was gassed with a mixture of 95% O_2, 5% CO_2.

[^{3}H]Vasopressin binding assays. Adenohypophyseal membranes (100–175 μg of protein) were incubated in 50 mM Tris-HCl (pH 7.4) containing $MgCl_2$ (5 mM), bovine serum albumin (1 mg/ml), [^{3}H]vasopressin (5 nM), and increasing amounts of unlabeled arginine vasopressin or vasopressin analogues (total volume 200 μl). Incubation was performed at 30° for 15 min. The reaction was initiated by the addition of membranes and stopped by addition of 4 ml of cold stopping solution (Tris-HCl, pH 7.4, 10 mM; $MgCl_2$, 1 mM) followed by immediate filtration through Millipore 0.45-μm filters and washing with 12 ml of the 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 arginine vasopressin and by five to six analogues was determined. Each peptide was tested at six different concentrations obtained by stepwise (1.63-fold) dilutions. The relative affinity of the analogue (arginine vasopressin used as a standard) was calculated from the ratio of IC_{50} values for arginine vasopressin and analogue. IC_{50} is the concentration of unlabeled peptide leading to a 50% inhibition of [^{3}H]vasopressin specific binding. All determinations were performed in triplicate, and the relative affinity of a given analogue was deduced from the results of three independent experiments (for details, see legend to fig. 1).

The binding assay on liver membranes was conducted as described previoulsy (30). Membranes (30 μg of protein) were incubated for 30 min at 30° in a total volume of 100 μl of a medium composed of Tris-HCl, pH 7.4, 50 mM; $MgCl_2$, 5 mM; bovine serum albumin, 1 mg/ml; [^{3}H]vasopressin, 5nM; and increasing amounts of the unlabeled peptide to be tested. The binding assay on kidney membranes was conducted as described in ref. 28. Membranes (100–200 μg of protein) were incubated for 15 min at 30° 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; [^{3}H]vasopressin, 5 nM; and increasing amounts of the unlabeled peptide to be tested. The competition experiments with liver and kidney membranes were conducted as indicated for experiments with rat hypophyseal membranes.

Bioassay of corticotropin-releasing activity. Cells were stimulated with 3-min pulses of the tested substance at 14-min intervals. Corticotropin releasing activity was measured by corticotropin release in excess of background (93 ± 7 pg of corticotropin release per min, 63 determinations). Corticotropin was monitored by radioimmunoassay (31) of 1-ml (2 min) fractions of cell column effluent. Each determination of anti-corticotropin-releasing activity was calculated from the results of four to seven experiments, each containing four to six pulses with AVP alone and four to six pulses with AVP given simultaneously with the tested analogue. The inhibition constant, K_i (the antagonist concentration eliciting a doubling of the apparent K_a value for arginine vasopressin) was deduced from the antagonist concentration leading to a 50% inhibition of the response elicited by 3 nM AVP (a value close

[1] G. Guillon *et al.*, unpublished observations.

TABLE 1

Structure and biological properties of the vasopressin analogues tested in the present study

References for the synthesis and pharmacological properties of the arginine vasopressin analogues listed in the table are given in Ref. 22 for (dEt_2VAVP), REf. 23 for d$(CH_2)_5$ [D-Tyr^2]VDAVP, and Ref. 24 for other arginine vasopressin analogues. pA_2 values were calculated from effective doses given in Ref. 24). The effective dose is defined as the dose (in nmol/kg) that reduces the response to *2x* units of agonist to equal the response to *1x* units of agonist. pA_2 values represent the negative logarithms of the effective dose divided by the estimated volume of distribution (67 ml/kg).

	ANALOGUE	Agonistic activities		Antagonistic activities	
		Antidiuretic	Vasopressor	Antidiuretic	Vasopressor
		units/mg		pA_2	
2[a]	desGly^{9}AVP[b]	164	0.05		6.05
16	d-Et_2VAVP				8.29
17	*d*$(CH_2)_5$[D-Tyr^2]VDAVP			7.03	8.05
4	d$(CH_2)_5$AVP	0.03			8.08
5	desGly^{9}d$(CH_2)_5$AVP	0.003			8.38
6	desGly$(NH_2)^9$*d*$(CH_2)_5$AVP	0.04			7.96
7	d$(CH_2)_5$[D-Phe^2]VAVP	weak[c]		8.0	8.06
8	desGly^{9}d$(CH_2)_5$[D-Phe^2]VAVP			8.06	8.15
9	desGly$(NH_2)^9$d$(CH_2)_5$[D-Phe^2]VAVP			7.71	7.92
10	d$(CH_2)_5$[Tyr(Et)^2]VAVP	0.03[c]		7.55	8.14
11	desGly^{9}d$(CH_2)_5$[Tyr(Et)^2]VAVP			7.83	8.17
12	d$(CH_2)_5$[D-Tyr(Et)^2]VAVP	weak[c]		7.78	8.17
13	desGly^{9}d$(CH_2)_5$[D-Tyr(Et)^2]VAVP			7.57	8.17
14	*d*$(CH_2)_5$[D-Phe^2,Ile^4]VAVP			8.16	7.83
15	desGly^{9}d$(CH_2)_5$[D-Phe^2,Ile^4)]AVP			8.01	7.83

[a] Numbers are the keys for symbols used in Figs. 3 and 4.

[b] Abbreviations: AVP, arginine vasopressin; desGly, desglycine; desGly(NH_2), desglycinamide; dEt_2VAVP, [1-(β-mercapto-β,β-diethylpropionic acid),4-valine] arginine vasopressin; d$(CH_2)_5$[D-Tyr^2]VDAVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-tyrosine,4-valine,8-D-arginine] vasopressin; d$(CH_2)_5$AVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid)] arginine vasopressin; d$(CH_2)_5$[D-Phe^2]VAVP, [1-β-(mercapto-β,β-cyclopentamethylenepropionic acid),2-D-phenylalanine,4-valine] arginine vasopressin; d$(CH_2)_5$[Tyr(Et)^2]VAVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-*O*-ethyityrosine, 4-valine] arginine vasopressin; d$(CH_2)_5$[D-Tyr(Et)^2]VAVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-(*O*-ethyl)tyrosine,4-valine] arginine vasopressin; d$(CH_2)_5$[D-Phe^2.Ile^4]AVP; [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-D-phenylalanine,4-isoleucine] arginine vasopressin.

[c] These analogues showed weak partial agonistic activity in these assays which was not clearly related to dose.

to the apparent K_a value: 4.3 ± 0.06 nM). The mean response to 3 nM AVP was 1900 ± 100 pg of corticotropin released per min (66 determinations).

Results and Discussion

None of the peptides tested (Table 1) which inhibited the *in vivo* vasopressor effect of vasopressin in rats was active in eliciting corticotropin release by isolated adenohypophyseal cells. They all inhibited [^{3}H]vasopressin binding to adenohypophyseal membranes in a dose-dependent manner (Fig. 1, *upper panel*). As expected, these peptides inhibited vasopressin-induced corticotropin release (Fig. 2). The data summarized in Table 2 indicated that there was a good correlation between the corresponding inhibition constants (K_i) determined on intact cells and the equilibrium dissociation constants (K_d) for binding to adenohypophyseal membranes. These results clearly confirm the conclusion (13) that the specific vasopressin-binding sites detected on adenohypophyseal membranes are the receptors mediating the vasopressin effect on corticotropin release. Indeed, the correlation between binding and biological activity now extends to a series of 21 vasopressin analogues (Fig. 3) including agonists and antagonists, the affinities of which are distributed over a very wide concentration range (more than 3 orders of magnitude).

One of the most salient facts derived from the present study is the observation (Fig. 4) that there was no correlation between the relative anti-corticotropin-releasing activities of the analogues tested and their antivasopressor or anti-antidiuretic potencies. This observation clearly indicates, as has already been suggested (16–18), that adenohypophyseal vasopressin receptors might have a ligand specificity different both from those of V_1 receptors involved in the pressor response and from V_2 receptors involved in the antidiuretic response.

To gain further insight into the problem we decided to compare the ligand specificities of adenohypophyseal and peripheral vasopressin receptors of the V_1 and V_2 types. This comparison was based on the determination of the equilibrium dissociation constants for vasopressin and vasopressin analogues binding to rat adenohypophyseal membranes, on the one hand, and to rat kidney and rat liver membranes, on the other (Fig. 1, Table 2).

The results obtained with liver membranes (Table 2) indicate that the cyclopentamethylene derivatives, d$(CH_2)_5$[D-Tyr^2]VDAVP, d$(CH_2)_5$[D-Phe^2]VAVP, d$(CH_2)_5$[Tyr(Et)^2]VAVP, d$(CH_2)_5$[D-Tyr(Et)^2]VAVP, and d$(CH_2)_5$[D-Phe^2, Ile^4]AVP, which are highly potent antivasopressor peptides *in vivo* (see Table 1), have high affinity for hepatic vasopressin receptors, close to or higher than that of arginine vasopressin itself. Deletion of the glycyl or glycinamide residue in position 9, which preserves a high antivasopressor potency (24), also preserves a high affinity for hepatic receptors. The correlation between antivasopressor potencies and affinity for liver membrane receptors also holds for d-Et_2VAVP and for desGly^{9}AVP. The former is a potent antivasopressor peptide and exhibits a high affinity for hepatic receptors, the latter is about 100 times less potent as an antivasopressor, and its affinity for hepatic receptors is reduced in a similar proportion. These data clearly confirm the conclusion (32, 33) that the ligand specificities of hepatic and vascular vasopressin receptors are very similar.

Similarly, comparison of antidiuretic or anti-antidiuretic activities of the tested analogues (Table 1) with the corresponding K_d values determined on rat kidney membranes (Table 2) reveals a good correspondence between the two sets of data. The

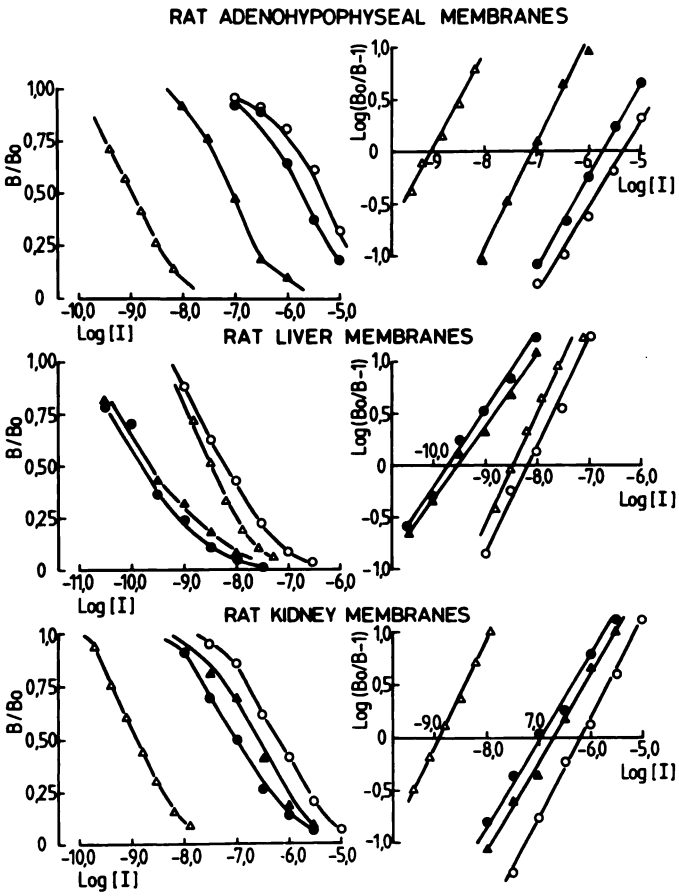

Fig. 1. Determination of the affinity of vasopressin analogues for rat hypophyseal, liver, and kidney membranes. The figure illustrates the procedure used for the determination of the affinity of unlabeled vasopressin analogues for rat adenohypophyseal (*upper panels*), rat liver (*middle panels*), and rat kidney membranes (*lower panels*). The competition experiments were conducted as indicated under Materials and Methods. *Left panels*. Dose-dependent inhibition curves. Data on the graph are means of nine individual values (triplicate determinations derived from three independent experiments). In each individual experiment dose-dependent inhibition of [^{3}H]vasopressin binding by unlabeled arginine vasopressin was determined. *Right panels*. Log $[(B/B_O) - 1]$ is plotted as a function of log $[I]$, in which B and B_O represent specific vasopressin binding in the presence and absence of unlabeled peptide, respectively: $[I]$ is the concentration of unlabeled peptide. The x-intercepts of the curves, (log $[K_d \times (1 + ([^3H]\text{vasopressin})/K_d[^3H]\text{vasopressin})]$, were calculated by linear regression analysis. The standard error was calculated as $S_{dx} = \sigma_x(1 - r^2)^{0.5}$, with r = correlation coefficient, and σ_x = SD of x values. The K_d values and the precision indexes given in Table 2 were deduced from these determinations.

highly potent antagonists (peptides 7–15) exhibit high affinity for kidney membranes while the weak agonists (peptides 4–6) exhibit low affinity. Altogether, data obtained on liver and kidney membranes validate the experimental approach used to demonstrate the existence of possible differences in the ligand specificities of adenohypophyseal, vascular, and renal vasopressin receptors.

Conversely, data shown in Table 2 clearly demonstrate that adenohypophyseal and hepatic vasopressin receptors have markedly different ligand specificities. In the series of vasopressin antagonists tested, it appears that: 1) deletion of the glycyl residue from the arginine vasopressin molecule is accompanied by an approximately 30 times greater reduction in affinity for adenohypophyseal receptors than for hepatic recep-

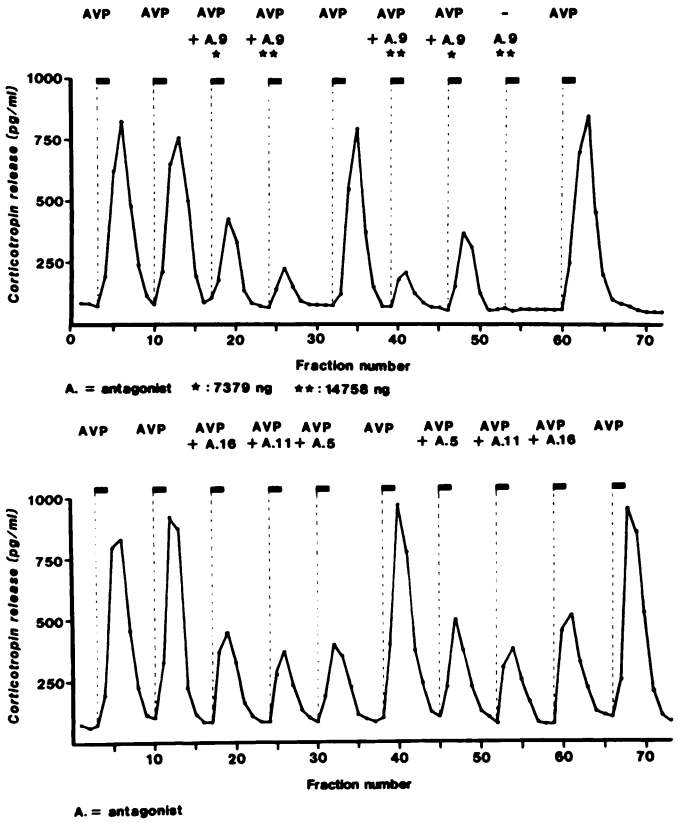

Fig. 2. Determination of anti-corticotropin-releasing activity of vasopressin antagonists. The figure illustrates parts of two typical experiments conducted as described under Materials and Methods. Corticotropin concentration in the perfusion medium is plotted as a function of time (each fraction corresponds to a 2-min perfusion period). ▬, the duration of the perfusion periods in the presence of the agents tested: vasopressin alone used at a constant concentration of 3 nM (AVP). vasopressin antagonists (*A*), and vasopressin plus antagonist. The magnitude of the response to a given 3-min perfusion of vasopressin, antagonist, or vasopressin plus antagonist, was measured by the total amount of corticotropin released over basal release during the 12-min period following the beginning of the perfusion. Note that vasopressin produced a rapid and reversible increase in corticotropin release and that corticotropin release returned to its basal level at the end of this 12-min period. Also note that the magnitude of the response to vasopressin remained fairly stable during the course of the experiment. *Upper panel*. The experiment shows that the antagonist desGly(NH$_2$)^{9}d(CH$_2$)$_5$[D-Phe^2]VAVP (A.9), when tested alone at a concentration of 14758 ng/ml (fractions 53–60), was completely inactive. It inhibited vasopressin-induced corticotropin release in a dose-dependent manner (compare the responses to AVP measured in the presence of 7379 and 14758 ng/ml respectively). *Lower panel*. A routine experiment in which the inhibitory effects of 3 antagonists were tested: d-Et$_2$VAVP (*A.16*), desGly^{9}d(CH$_2$)$_5$[Tyr(Et)^2]VAVP (*A.11*), and desGly^{9}d(CH$_2$)$_5$AVP (*A.5*). The antagonists were used at concentrations equal to their respective K_d values determined on adenohypophyseal membranes (see Fig. 1 and Table 2). AVP was used at a 3 nM concentration. The fractional inhibition (X = response in the presence of antagonist/response in absence of antagonist) was determined by reference to the mean of the responses to vasopressin determined before and after the application of vasopressin plus antagonist. The inhibition constant (K_i) was calculated as:

$$K_i = [I] \times [X/(1 + [\text{AVP}]/K_a)(1 - X)]$$

in which $[I]$ and [AVP] are the concentrations of vasopressin and antagonist, respectively, and K_a is the vasopressin concentration eliciting half-maximal corticotropin release. K_i values were determined four to nine times using different cell preparations. The mean values ± SD are given in Table 2.

TABLE 2

Ligand specificities of rat adenohypophyseal, hepatic, and renal vasopressin receptors

K_i values for the inhibition of vasopressin-induced corticotropin release were determined as indicated in the legend to Fig. 2. Values are means ± SD for the number of determinations indicated in parentheses. K_d values were deduced from experiments similar to those illustrated by Fig. 1. K_i and K_d values are expressed in nM. The precision index was calculated as indicated in the legend to Fig. 1. The selectivity indexes indicated in the last two columns are defined as the ratio of relative affinities using AVP as reference ($K_{d\ analogue}/K_{d\ AVP}$). The values of $K_{d\ AVP}$ used were 1.45 ± 0.31, 3.47 ± 0.22, and 1.02 ± 0.15 nM for kidney, liver, and adenohypophyseal membranes, respectively. The correlation coefficient between K_i (anti-corticotropin releasing activity) and K_d values for binding to adenohypophyseal membranes was $r = 0.736$, $p < 0.01$.

	ANTAGONIST TESTED	K_i hypophyseal cells	K_d hypophyseal membranes (A)	K_d liver membranes (B)	K_d kidney membranes (C)	Selectivity index A/B	Selectivity index A/C
2*	desGly^{9}AVP	5,469 ± 371 (5)	3,090 ± 406	372 ± 55	3.2 ± 0.7	29	1385
16	d-Et$_2$VAVP	130 ± 14 (7)	18 ± 2	2.9 ± 0.2	5.5 ± 0.9	20	5
17	d(CH$_2$)$_5$[D-Tyr^2]VDAVP	551 ± 55 (5)	355 ± 104	8 ± 1	3.2 ± 0.6	150	160
4	d(CH$_2$)$_5$AVP	1,016 ± 185 (5)	117 ± 27	0.3 ± 0.1	190 ± 25	1,300	0.9
5	desGly^{9}d(CH$_2$)$_5$AVP	704 ± 133 (4)	1,905 ± 105	0.23 ± 0.05	110 ± 23	29,240	25
6	desGly(NH$_2$)^{9}d(CH$_2$)$_5$AVP	4,238 ± 363 (7)	5,130 ± 900	7.2 ± 0.9	660 ± 70	29,240	11
7	d(CH$_2$)$_5$[D-Phe^2]VAVP	248 ± 32 (9)	81 ± 8	4.7 ± 0.6	0.3 ± 0.1	62	410
8	desGly^{9}d(CH$_2$)$_5$[D-Phe^2]VAVP	667 ± 211 (9)	977 ± 13	2.5 ± 0.5	0.22 ± 0.08	1,340	6,300
9	desGly(NH$_2$)^{9}d(CH$_2$)$_5$[D-Phe^2] VAVP	3,042 ± 492 (5)	9,300 ± 2,500	4.0 ± 0.6	0.4 ± 0.2	8,200	28,750
10	d(CH$_2$)$_5$[Tyr(Et^2)]VAVP	1,403 ± 310 (4)	1,513 ± 136	0.3 ± 0.1	0.3 ± 0.1	17,340	8,250
11	desGly^{9}d(CH$_2$)$_5$[Tyr(Et^2)]VAVP	4,763 ± 712 (4)	15,000 ± 1,900	0.2 ± 0.1	0.21 ± 0.09	260,600	102,000
12	d(CH$_2$)$_5$[D-Tyr(Et^2)]VAVP	570 ± 225 (7)	51 ± 6	0.14 ± 0.06	0.17 ± 0.09	1,290	425
13	desGly^{9}d(CH$_2$)$_5$[D-Tyr(Et^2)]VAVP	2,079 ± 416 (5)	2,040 ± 350	0.19 ± 0.08	0.12 ± 0.04	38,000	24,100
14	d(CH$_2$)$_5$[D-Phe^2,Ile^4]VAVP	105 ± 17 (4)	43 ± 10	7 ± 1	0.4 ± 0.1	20	140
15	desGly^{9}d(CH$_2$)$_5$[D-Phe^2,Ile^4)]AVP	594 ± 75 (9)	871 ± 128	4.9 ± 0.6	0.7 ± 0.2	607	1,580

* Numbers are the key for symbols used in Figs. 3 and 4.

tors, and 2) substitutions on the β carbon of β-mercaptopropionic acid at position 1 clearly have different effects on the two receptor types. The diethyl substituent in d-Et$_2$VAVP and the cyclopentamethylene substituent in d(CH$_2$)$_5$AVP lead to selectivity indexes of 20 and 1,300 respectively. 3) deletion of the glycyl residue from the cyclopentamethylene-substituted analogues increases the selectivity index more than 10-fold (compare peptides 4–5, 7–8, 10–11, 12–13, 14–15). Deletion of the glycinamide residue leads to a further increase in the selectivity index in the case of d(CH$_2$)$_5$[D-Phe^2]VAVP but not in the case of d(CH$_2$)$_5$AVP. 4) It is clear also that the L-Tyr(Et)-containing analogues exhibit a significant enhancement in their selectivity indexes relative to their diastereoisomeric D-Tyr(Et)-containing counterparts. Thus, d(CH$_2$)$_5$[Tyr(Et)^2]VAVP has one of the highest selectivity indexes (17,340). Deletion of the glycyl residue in this peptide leads to a further enhancement to 260,000 of the selectivity index. The D-Tyr(Et)^2 derivatives, although still highly selective, are much less so than their L-Tyr(Et)^2-containing counterparts (compare peptides 10–4 to 12–13).

The ligand specificity of adenohypophyseal receptors is also markedly different from that of V_2 renal receptors. For peptides which are antagonists of both the antidiuretic and vasopressor responses (peptides 7–15, 17) the selectivity indexes, hypophysis versus liver and hypophysis versus kidney, are roughly comparable. It is interesting to note that the peptides which discriminate the less efficiently between hypophyseal and renal receptors are, except for desGly^{9}AVP, those which exhibit weak antidiuretic activity (peptides 4–6).

The present study provides a direct demonstration that vasopressin receptors mediating vasopressin-induced corticotropin release represent a novel type of vasopressin receptor that is quite distinct from the previously characterized peripheral vasopressin receptors. The characterization of this new type of vasopressin receptor was rendered possible by the use of vasopressin antagonists designed as potent antivasopressor and anti-antidiuretic peptides. These antagonists have markedly decreased affinities for adenohypophyseal receptors. Incidentally, those observations might account for some of the discrepancies between studies using this type of antagonist to investigate the physiological relevance of the vasopressin action on corticotropin release (35–36). Our results now raise the problem of designing potent agonists and antagonists which are selective for adenohypophyseal vasopressin receptors. We have shown that the structural modifications of the vasopressin molecule which confer antivasopressor or anti-antidiuretic properties to the resulting peptides also confer anti-corticotropin-releasing activity. Although none of the antagonists tested exhibited detectable corticotropin-releasing activity, we cannot exclude that some of them might be partial agonists with very low intrinsic activity. Indeed, it was recently reported that in the presence of physiological concentrations of corticotropin-releasing factor, several vasopressin antagonists, including [1-(β-mercapto-β,β-cyclopentamethylene propionicacid), 2-*O*-methyl-tyrosine] arginine vasopressin, the parent compound of the antagonists used in the present study, are partial agonists (19). It would be of interest to examine whether the new antagonists tested have any agonistic properties in the presence of corticotropin-releasing factor. This information is of importance since it concerns the potential practical application of these antagonists *in vivo*.

As far as the definition of adenohypophyseal receptors in terms of V_1, V_2, an additional type of vasopressin receptors is concerned, one has to keep in mind that, according to the initial proposal of Michell *et al.* (9), the definition of V_1 and V_2 receptors must fulfill both functional and pharmacological criteria. Strictly speaking, adenohypophyseal receptors cannot be considered as V_1 or V_2 receptors since their ligand specificity is clearly distinct from those of classical V_1 and V_2 receptors. From the functional viewpoint there is, as previously indicated, experimental evidence[1] (14) that adenohypophyseal receptors are not coupled to adenylate cyclase but, more probably, to the

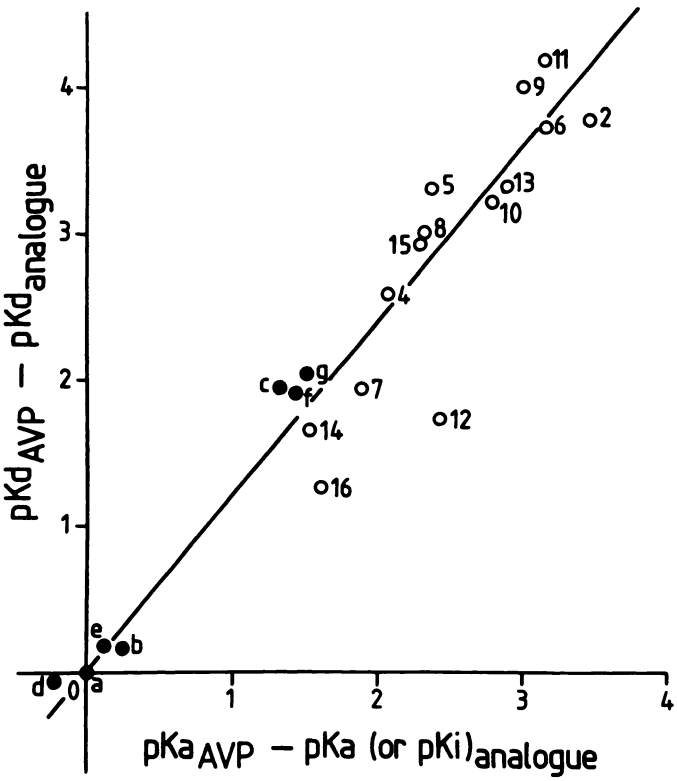

Fig. 3. Correlation between the affinities of some vasopressin analogues for adenohypophyseal membranes and the corresponding corticotropin-releasing or anti-corticotropin-releasing potencies. Results from the binding experiments are expressed as $pK_{d\ AVP} - pK_{d\ analogue}$ (i.e., as the negative logarithm of relative affinity). Similarly, the corresponding biological potencies of the tested analogues are expressed as $(pK_{a\ AVP} - pK_a$ (or $pK_i)_{analogue}$). The figure includes the data obtained in a previous study (13) using a series of vasopressin agonists. The relative affinity for adenohypophyseal membranes $(pK_{d\ AVP} - pK_{d\ analogue})$ is plotted as a function of the logarithm of the relative biological potency $(pK_{a\ AVP} - pK_a$ (or $pK_i)_{analogue}$). ●, data obtained with agonists; ○, data obtained with antagonists. The correlation between the two sets of data is highly significant ($r = 0.960$, $p < 0.001$). The equation of the linear regression line is: $y = 1.181x - 0.001$. *a*, arginine vasopressin; *b*, lysine vasopressin; *c*, oxytocin; *d*, [1-(L-α-hydroxy-β-mercaptopropionic acid)] arginine vasopressin; *e*, [2-phenylalanine, 8-ornithine] vasotocin (or oxytocin); *f*, [1-(L-α-hydroxy-β-mercaptopropionic acid),4-valine,8-D-arginine] vasopressin; g, 1-deamino-[8-D-arginine] vasopressin. The key to the *numbers* is given in the legend to Table 1.

transduction mechanism leading to calcium mobilization through an increased phosphatidylinositol 4,5-bisphosphate breakdown. Based on these conclusions, we tentatively propose that adenohypophyseal vasopressin receptors might represent a subtype of V_1 receptors which could be designated as V_{1b} receptors as opposed to V_{1a} receptors clearly characterized on liver and blood vessels. The use of vasopressin antagonists of the series tested in the present study could represent appropriate tools for discriminating between V_{1a} and V_{1b} receptors.

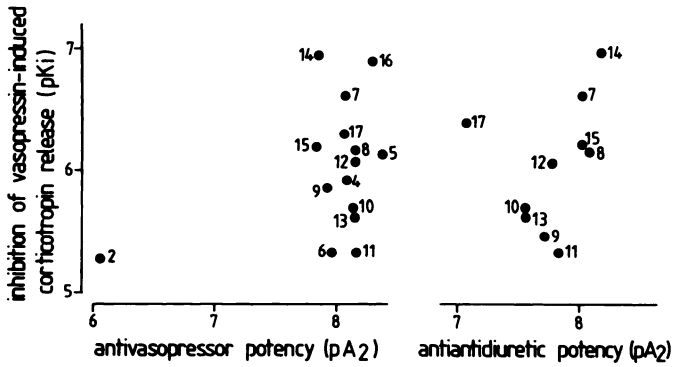

Fig. 4. Absence of correlation between anti-corticotropin-releasing potencies of vasopressin antagonists and the corresponding antivasopressor and anti-antidiuretic potencies. The figure was constructed using the antivasopressor and anti-antidiuretic potencies given in Table 1 and the anti-corticotropin-releasing activities determined in the present study (see Table 2). Antivasopressor and anti-antidiuretic activities are expressed in terms of pA_2 values (see legend to Table 1). The key to symbols is given in Table 2. Linear regression analysis of the data gave *r* values of 0.341 (anti-corticotropin-releasing versus antivasopressor potencies), and 0.340 (anti-corticotropin-releasing versus anti-antidiuretic potencies). These correlations are not statistically significant.

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