MOLECULAR PHARMACOLOGY, 14, 1006-1017

Structural Requirements for Activation of Vasopressin-sensitive Adenylate Cyclase, Hormone Binding, and Antidiuretic Actions: Effects of Highly Potent Analogues and Competitive Inhibitors

DANIEL BUTLEN, GILLES GUILLON, RABARY M. RAJERISON, AND SERGE JARD

Laboratoire de Physiologie Cellulaire, Collège de France, 75231 Paris Cedex 05, France

WILBUR H. SAWYER

Department of Pharmacology, College of Physicians and Surgeons of Columbia University, New York, New York 10032

MAURICE MANNING

Department of Biochemistry, Medical College of Ohio, Toledo, Ohio 43699

(Received May 8, 1978)
(Accepted July 17, 1978)

SUMMARY

BUTLEN, D., GUILLON, G., RAJERISON, R. M., JARD, S., SAWYER, W. H., & MANNING, M. (1978) Structural requirements for activation of vasopressin-sensitive adenylate cyclase, hormone binding, and antidiuretic actions: Effects of highly potent analogues and competitive inhibitors. *Mol. Pharmacol.*, **14**, 1006-1017.

Membranes prepared from the medullopapillary portion of pig and rat kidneys were used to test the relative abilities of 11 arginine-vasopressin structural analogues to activate adenylate cyclase and to inhibit [^{3}H]lysine-vasopressin binding to these membrane preparations. The analogues tested were: [1-deaminopenicillamine, 2-O-methyltyrosine]-arginine-vasopressin; [1-deaminopenicillamine]arginine-vasopressin; [1-deaminopenicillamine, 4 valine, 8-D-arginine]-vasopressin (dP-V-DAVP); [8-D-arginine]-vasopressin; [2-O-methyltyrosine]arginine-vasopressin; [4-valine, 8-D-arginine]-vasopressin; [4-valine]arginine-vasopressin; deamino [4-threonine, 8-D-arginine]-vasopressin; deamino [8-D-arginine]-vasopressin; deamino [4-valine, 8-D-arginine]-vasopressin; [1(β-mercapto-β,β-cyclopentamethylene propionic acid), 4-valine, 8-D-arginine]-vasopressin (cyclo-dV-DAVP). Cyclo-dV-DAVP behaved like a competitive inhibitor of vasopressin-induced adenylate cyclase activation. The apparent K_i values were 10 and 310 nM for the rat and pig systems, respectively. This peptide is the most active antagonist described so far. dP-V-DAVP behaved like a competitive inhibitor on the pig system (K_i = 2.9 μM) and was found to produce a maximal enzyme activation that was 75% of that induced by arginine-vasopressin in the rat system (K_{act} = 3.7 nM). In both the rat and pig systems there was a good correlation between the K_{bind} values for binding to renal membranes and the corresponding K_{act} or K_i values for the adenylate cyclase response. The structural requirements for binding to the pig renal receptor and to the rat renal receptor were found to be different. When one takes into account for metabolic stability of the ADH analogues tested (as estimated by the relative duration of the antidiuretic response), there was a satisfactory correlation between the antidiuretic activities of the peptides tested in the rat and their abilities to activate the adenylate cyclase present on renal membranes.

This work was supported by the Centre National de la Recherche Scientifique (L.A. 219) and by the Délégation Générale à la Recherche Scientifique et Technique (grant 77.7.1277.01).

0026-895X/78/0146-1006$02.00/0
Copyright © 1978 by Academic Press, Inc.
All rights of reproduction in any form reserved.

INTRODUCTION

Previous studies have indicated that the antidiuretic hormone-sensitive adenylate cyclase from the kidney of several mammalian species is able to discriminate among neurohypophysial peptides and analogues with closely related structures (1–7). Rough correlations exist between the relative abilities of these peptides to activate renal adenylate cylase and their relative abilities to induce an antidiuretic response in the intact animal. Thus among the three natural neurohypophysial peptides in mammals—arginine-vasopressin, lysine-vasopressin, and oxytocin—the most potent antidiuretic peptide in the rat, arginine-vasopressin, is also the most potent activator of rat renal adenylate cyclase (8, 9). Oxytocin, which has poor antidiuretic activity, is much less active on renal adenylate cyclase than either arginine- or lysine-vasopressin. However, marked discrepancies between data *in vitro* and *in vivo* were observed. First, the hormone concentrations needed to elicit maximal adenylate cyclase activation *in vitro* were found to be much higher than blood hormone concentrations eliciting a full antidiuretic response (10). It was suggested that the so-called receptor reserve phenomenon is highly operative in the kidney, i.e., it was suggested that occupancy of a small fraction of the total number of hormone receptors is sufficient to induce a full antidiuretic response. Such an hypothesis might explain the observation that peptides that have very low abilities to activate the adenylate cyclase system (2, 4, 5) are able to elicit a full antidiuretic response (11, 12). In fact, among the very large series of vasopressin analogues that have been synthesized, none has been found to be a potent competitive inhibitor of the antidiuretic action of vasopressin. In addition, vasopressin analogues like deamino [8-D-arginine]-vasopressin, which has a very high antidiuretic activity in the rat, is no more efficient than arginine-vasopressin in activating rat renal adenylate cyclase (7). Structural modifications that lead to enhanced antidiuretic activity may also enhance metabolic stability of the peptide *in vivo* (such as deamination, substitution of D-aminoacids for L-aminoacids, increase in the overall hydrophobicity of the molecule, etc.). Therefore, it was suggested that for these analogues the high antidiuretic potency was mainly related to greater metabolic stability (13–19).[1]

In the present study, we report the measurement of kinetic parameters of the effects of a series of arginine-vasopressin analogues on the hormone-sensitive adenylate cyclases from rat and pig kidney. The series of analogues tested included peptides of high and selective antidiuretic potency, and peptides that were shown to be inhibitors of the vascular effect of vasopressin *in vivo*. It will be shown that when one takes the metabolic stability *in vivo* of these peptides into account a good correlation between data *in vivo* and *in vitro* can be demonstrated. Furthermore the properties of a highly potent inhibitor of arginine-vasopressin-induced adenylate cyclase activation from rat and pig kidneys will be described.

MATERIALS AND METHODS

Materials. The arginine-vasopressin analogues used in this study are listed in Table 1. Their syntheses and pharmacological properties have been reported elsewhere (13–19).[1] Tritiated lysine-vasopressin ([^{3}H]Tyr^2-Lys^8-vasopressin) was prepared according to a previously described procedure (21). The labeled peptide was purified by affinity chromatography using neurophysin bound to sepharose 4B. Its specific radioactivity was 10.8 Ci/mmol. Its biological activity determined on the rat vasopressor assay (10) and rat and pig kidney adenylate cyclase assays was identical to that of the starting material (synthetic Lys^8-vasopressin from Bachem). Other chemicals were obtained from the following sources: neutral aluminium oxide from Woelm (Eschwege); DOWEX AG50WX8 from Biorad Laboratories; sodium dodecyl sulfate from Serlabo; bovine serum albumin fraction V from Pentex; Tris and ATP (disodium salt) from Sigma Chemical Company; cyclic AMP, creatine kinase and phosphocreatine (disodium salt) from Boehringer; EDTA, ouabain and sodium azide from Merck; [^{3}H]cyclic AMP (21 Ci/mmol) from Commissariat à l'Energie

[1] Bankowski, K., Manning, M., Haldar, J. & Sawyer W. H, *J. Med. Chem.*, in press.

TABLE 1

List of compounds

Compounds	Abbreviation	Antidiuretic[a] activity	References for synthesis
		IU/mg	
[8-arginine]-vasopressin[b]	AVP	—	
[1-deaminopenicillamine, 2-O-methyltyrosine]-arginine-vasopressin	dP-Tyr(OMe)AVP	3.5	Footnote 1
[1-deaminopenicillamine]arginine-vasopressin	dP-AVP	42.0	Footnote 1
[1-deaminopenicillamine, 4 valine, 8-D-arginine]-vasopressin	dP-V-DAVP	160.0	(18)
[8-D-arginine]-vasopressin	DAVP	257.0	(17)
[2-O-methyltyrosine]arginine-vasopressin	Tyr(OMe)AVP	450.0	Footnote 1
[4-valine, 8-D-arginine]-vasopressin	V-DAVP	653.0	(14)
[4-valine]arginine-vasopressin	V-AVP	738.0	(14)
deamino[4-threonine, 8-D-arginine]-vasopressin	dT-DAVP	793.0	(17)
deamino[8-D-arginine]-vasopressin	d-DAVP	955.0	(17)
deamino[4-valine, 8-D-arginine]-vasopressin	dV-DAVP	1230.0	(13)
[1(β-mercapto-β, β-cyclopentamethylene propionic acid), 4-valine, 8-D-arginine]-vasopressin	cyclo dV-DAVP	0.01	(19)

[a] Values determined on the rat system (14–15) and unpublished data.

[b] 8-arginine-vasopressin was isolated from bovine neurophysin complex as described by Prusík *et al.* (20). It was provided by Dr. T. Barth, to whom we are indebted.

Atomique, Saclay, France, [α-^{32}P]ATP (20 Ci/mmol) from New England Nuclear.

Membrane preparation. Membrane fractions were prepared from the medullopapillary portions of pig and Wistar rat kidneys, according to previously described procedures (3, 8).

Adenylate cyclase assay. Adenylate cyclase activity was measured by conversion of [α-^{32}P]ATP into labeled cyclic AMP. The incubation medium (100 μl final volume) contained: Tris-HCl 100 μM, pH 7.4 (rat) or pH 8.0 (pig); $MgCl_2$, 0.75 mM; EDTA-Tris 0.25 mM; cyclic AMP, 1 mM; creatine phosphate, 20 mM; creatine kinase, 100 μg; ouabain, 0.1 mM; sodium azide, 10 mM; and various amounts of arginine-vasopressin or analogues. Membranes (100–200 μg protein) were incubated for 15 min at 30° in the above described medium. The reaction was initiated by the addition of substrate ATP 0.25 mM and [α-^{32}P]ATP about 0.65 μCi; it was allowed to proceed for 6 min at 30° and stopped by addition of sodium dodecyl sulfate (2% final concentration). Labeled cyclic AMP was separated by the method of Salomon *et al.* (22) with minor modifications. Cyclic AMP recovery was monitored using [^{3}H]cyclic AMP added immediately after stopping the reaction. The dose dependency for adenylate cyclase activation by vasopressin and analogues was characterized by: the maximal increase in enzyme activity over basal value (V_{max}), K_{act} (concentration of peptide leading to half maximal activation), and the Hill coefficient. Proteins were determined by Lowry's method (23) using bovine serum albumin as a standard.

Binding assay. Binding of [^{3}H]lysine-vasopressin was measured under experimental conditions identical to those used for the adenylate cyclase assay, except for the absence of [α-^{32}P]ATP in the incubation medium. After 18 min incubation in the presence of [^{3}H]lysine-vasopressin, the incubation medium was diluted by 2 ml of a cold solution containing Tris-HCl, 25 mM, pH 7.4, and $MgCl_2$, 0.75 mM. Bound radioactivity was separated by filtration on millipore filters EAWP 1 μm. The filter was washed three times with 10 ml of a cold Tris-HCl and $MgCl_2$ solution. All determinations were corrected for nonspecific binding, i.e., residual radioactivity measured in the presence of a large excess of unlabeled lysine-vasopressin (5 μM). The binding curve for lysine-vasopressin was deter-

mined using increasing amounts of the labeled peptide. The binding constant (K_{bind}) was calculated as the concentration of peptide leading to half maximal specific binding. The binding constants for unlabeled vasopressin and analogues were determined from competition experiments. Membranes were incubated in the presence of a constant amount of [^{3}H]lysine-vasopressin (2.5–11.1 nM) and increasing amounts of unlabeled peptide. K_{bind} values were calculated from the concentration (I_{50}) of unlabeled peptide leading to 50% inhibition of specific [^{3}H]lysine-vasopressin binding. Each experiment consisted of the determination of the dose-dependent [^{3}H]lysine-vasopressin binding, dose-dependent inhibition of [^{3}H]lysine-vasopressin binding by unlabeled arginine-vasopressin and analogues, and the corresponding dose-dependent adenylate cyclase activation curves.

RESULTS

The dose-dependencies for [^{3}H]lysine-vasopressin binding and adenylate cyclase activation by the labeled peptide from two typical experiments performed on rat and pig kidney membranes are depicted in Fig. 1. Characteristics of vasopressin binding and hormone sensitive adenylate cyclase (means ± S.E.) determined from several experiments similar to those described above, are in agreement with previously reported results (3–5, 8, 9). The dissociation constant for [^{3}H]lysine-vasopressin binding to rat kidney membranes was K_{bind} = 4.2 ± 0.7 nM, maximal binding capacity (B_{max}) was 0.22 ± 0.02 pmol [^{3}H]lysine-vasopressin bound/mg protein; the binding curve is noncooperative (Hill coefficient n = 1.03 ± 0.03, 10 determinations). The corresponding values for adenylate cyclase activation were K_{act} = 3.5 ± 0.4 nM, maximal activation (V_{max}) = 239 ± 58 pmol cyclic AMP/6 min/mg protein, Hill coefficient n = 0.86 ± 0.04 (9 determinations). Values obtained from experiments performed with pig kidney membranes are the following: K_{bind} = 11.2 ± 1.0 nM; B_{max} = 1.19 ± 0.30 pmol [^{3}H]lysine-vasopressin bound/mg protein, n = 1.35 ± 0.25; K_{act} = 4.0 ± 1.1 nM; V_{max} = 181 ± 20 pmol cyclic AMP/6 min/mg protein, n = 0.48 ± 0.03 (3 determinations). Comparison of dose dependencies for adenylate cyclase activation and hormone binding indicated the existence of a nonlinear relationship between response and receptor occupancy, as previously reported (3–5).

Figure 2 illustrates the results of a typical experiment with arginine-vasopressin and dV-DAVP. These two peptides were able to inhibit [^{3}H]lysine-vasopressin binding to the same maximal extent. The dissociation constants for binding to membranes were calculated from the I_{50} values as indicated under MATERIALS AND METHODS. For both the rat and pig systems the dissociation constants found were close to corresponding K_{act} values for adenylate cyclase activation. The pig and rat systems have different stereospecificities. Thus, in the rat K_{act} for dV-DAVP was close to that for arginine-vasopressin (0.45 compared to 0.35 nM). For the porcine system dV-DAVP is about 1,000 times less active than arginine-vasopressin. Maximal adenylate cyclase activation induced by dV-DAVP was higher than that induced by arginine-vasopressin in the rat system; the reverse situation was observed for the porcine system.

Results obtained with the entire series of analogues tested are given in Table 2. From these results, it is clear that the rat renal receptor discriminated much less efficiently among arginine-vasopressin analogues than did the pig renal receptor. The extreme K_{bind} values measured were 0.22 nM (for V-DAVP) and 32 nM (for dP-Tyr (OMe)AVP) in the rat. In the pig system the extreme K_{bind} values were 1.73 nM (for arginine-vasopressin) and 3060 nM (for d-DAVP). Only V-AVP was able to induce a maximal adenylate cyclase activation higher than that induced by arginine-vasopressin in the pig kidney system. Several analogues (dV-DAVP, V-DAVP, Tyr (OMe)AVP, and dP-AVP) had V_{max} values higher than that of arginine-vasopressin on the rat system.

Cyclo-dV-DAVP was unable to activate the adenylate cyclases from either the pig and rat kidneys. dP-V-DAVP had a very low V_{max} value in the pig but was almost as active as arginine-vasopressin in the rat. These two peptides were able to inhibit [^{3}H]lysine-vasopressin binding in both the

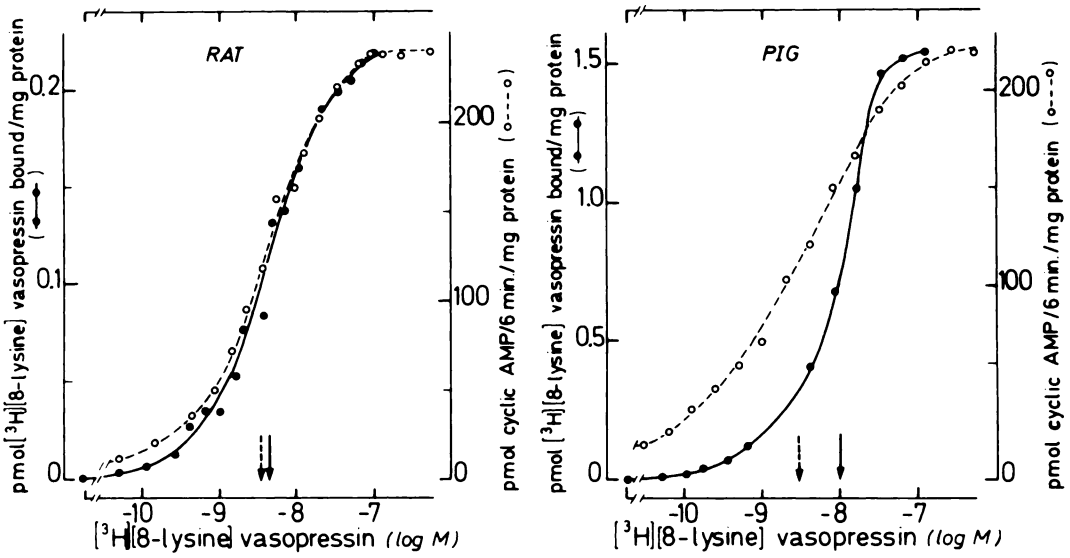

FIG. 1. *Binding of [³H]lysine-vasopressin to renal membranes and activation of adenylate cyclase by the labeled peptide*

The figure illustrates the results of 2 typical experiments: one was performed on membranes prepared from rat kidney (left panel), the other on membranes prepared from pig kidney (right panel). The binding of [³H]-lysine-vasopressin and adenylate cyclase activation by labeled peptide were measured under identical experimental conditions as indicated in MATERIAL AND METHODS. Specific binding was measured by the difference between total binding determined with the indicated amounts of [³H]lysine-vasopressin and nonspecific binding measured in presence of 5 μM of unlabeled lysine-vasopressin. Adenylate cyclase activation was measured by the difference between the activity measured in presence of [³H]lysine-vasopressin and basal activity, i.e., activity measured in the absence of added hormone. Basal activities were 86 and 37 pmol cyclic AMP/6 min/mg protein for the rat (left panel) and the pig (right panel) systems respectively. Arrows on the graph indicate K_{bind} for hormonal binding and K_{act} for adenylate cyclase activation.

two systems. As shown in Fig. 3, cyclo-dV-DAVP behaved like an inhibitor of adenylate cyclase activation by lysine-vasopressin. The inhibition was of a competitive type in the rat system. Maximal activation in the presence of inhibitor was almost identical to that measured in its absence. In the presence of cyclo-dV-DAVP, the Hill coefficient of the activation curve was increased as compared to that obtained on the control preparation. In the pig system the inhibition by cyclo-dV-DAVP of adenylate cyclase activation by lysine-vasopressin was of a mixed type: the V_{max} value was decreased in the presence of inhibitor and the apparent K_{act} shifted toward higher values. The Hill coefficient of the activation curve was increased by the inhibitor. In the pig system dP-V-DAVP inhibited adenylate cyclase activation by lysine-vasopressin. The inhibition observed (Fig. 4) had the same characteristics as that described for cyclo-dV-DAVP. The dose-dependencies for adenylate cyclase activation in the absence or presence of inhibiting peptides were not of a michaelian type. Therefore it was not possible to calculate the apparent inhibition constants (K_i) in the classical manner for these peptides. We found that the dose-dependent activation of adenylate cyclase can be adequately described by the following relation:

$$V = V_{max} \times [H]^{n_i}/([H]^{n_i} + (K_{act})^n \cdot (1 + ([I]/K_i)^m))$$

where [H] = concentration of active peptide; n_i = Hill coefficient of the dose-response relationship in presence of inhibitor; n = Hill coefficient of the dose-response relationship in absence of inhibitor; [I] = concentration of inhibitor; m is a constant depending on the nature of the inhibitor.

From the above relation, it can be deduced that for [H] = A_{50} (i.e., the active peptide concentration leading to half max-

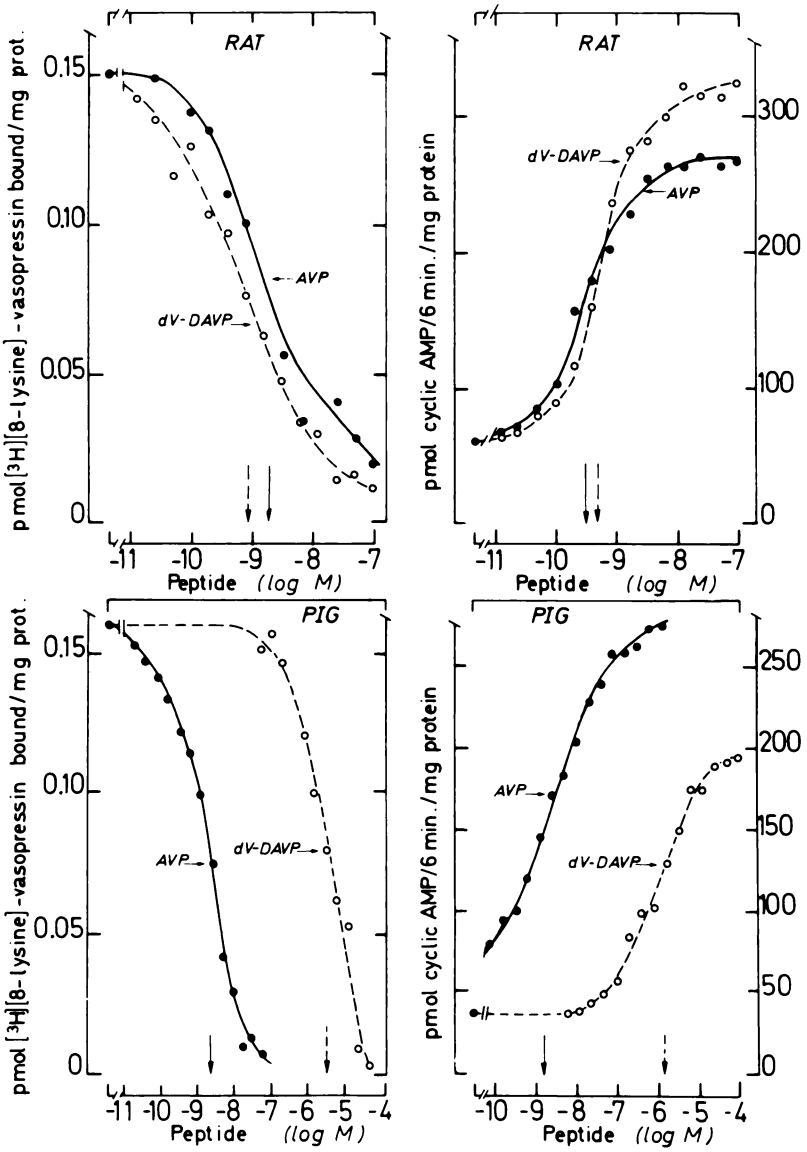

FIG. 2. *Estimation and K_{bind} of K_{act} values for [8-arginine]-vasopressin and deamino [4-valine, 8-D-arginine]-vasopressin in the rat and pig systems*

The figure illustrates the results of 2 typical experiments. One was performed on membrane prepared from the rat kidney (upper panels). The other on membrane prepared from pig kidney (lower panels). Dose-dependent adenylate cyclase activation by the two peptides tested was measured as indicated under MATERIALS AND METHODS. Arrows on the graph (right panels) indicate the experimental K_{act} values. K_{bind} values were deduced from competition experiments depicted in the left part of figure. Membranes were incubated in presence of a constant amount of [^{3}H]lysine-vasopressin: 10 nM (rat) and 2.5 nM (pig), and the indicated amounts of unlabeled peptides. All values on the graph are corrected for nonspecific binding (see MATERIALS AND METHODS). Arrows on the graph (left panels) indicate I_{50} values, i.e., concentration of unlabeled peptide leading to 50% inhibition of [^{3}H]lysine-vasopressin specific binding. The corresponding K_{bind} values were calculated using the following relation:

$$K_{bind} = I_{50} \times \frac{K_{bind}\,[^3H]LVP}{([^3H]LVP) + K_{bind}\,[^3H]LVP}$$

The value of K_{bind} [^{3}H]LVP (lysine-vasopressin) used for the calculation is that determined from a dose-dependent [^{3}H]lysine-vasopressin specific binding curve similar to that described in Fig. 1. Experimental values obtained were: K_{bind} [^{3}H]LVP = 10 nM, $K_{bind\ AVP}$ = 1.73 nM, $K_{bind\ dV\text{-}DAVP}$ = 2.44 μM for the pig system; the corresponding values for the rat system were 4.16 nM, 0.55 nM and 0.26 nM.

TABLE 2

Kinetic parameters for binding and for adenylate cyclase activation

Compounds[a]	Rat				Pig			
	K_{bind}	K_{act}	$\frac{V_{max}\text{ analogue}}{V_{max}\text{ AVP}} \times 100$	Hill coeff	K_{bind}	K_{act}	$\frac{V_{max}\text{ analogue}}{V_{max}\text{ AVP}} \times 100$	Hill coeff
	nM	*nM*			*nM*	*nM*		
AVP[b]	0.39 ± 0.09 (3)[b]	0.35 ± 0.06 (3)[b]	100	0.79 ± 0.01 (3)[b]	1.73	0.99 ± 0.45 (3)[b]	100	0.60 ± 0.04 (3)[b]
V-DAVP	0.22	0.33	120	1.22	2740	2510	76	0.81
dV-DAVP	0.26	0.45	123	0.80	2440	1410	73	0.80
dT-DAVP	0.24	0.43	94	0.78	470	930	67	0.74
d-DAVP	0.28	0.28	88	1.00	3060	3450	100	1.02
V-AVP	0.30	0.12	73	0.94	35.8	43.0	112	0.55
DAVP	0.54	0.48	96	0.67	1420	2850	65	0.72
Tyr (OMe)AVP	1.03	13.60	193	0.46	21.4	18.5	98	1.00
dP-AVP	14.50	21.50	189	0.61	250	630	71	0.80
dP-Tyr(OMe)AVP	31.10	15.10	78	0.65	730	1850	38	0.60
dP-V-DAVP	1.39	3.69	75	0.80	640	$K_i = 2900$[c]	7	—
Cyclo-dV-DAVP	12.00	$K_i = 10.00$	7	—	130	$K_i = 310$[c]	3	—

[a] Abbreviations of compounds explained in Table 1.

[b] Means ± S.E. of 3 individual experimental determinations.

[c] Means of 2 individual experimental determinations.

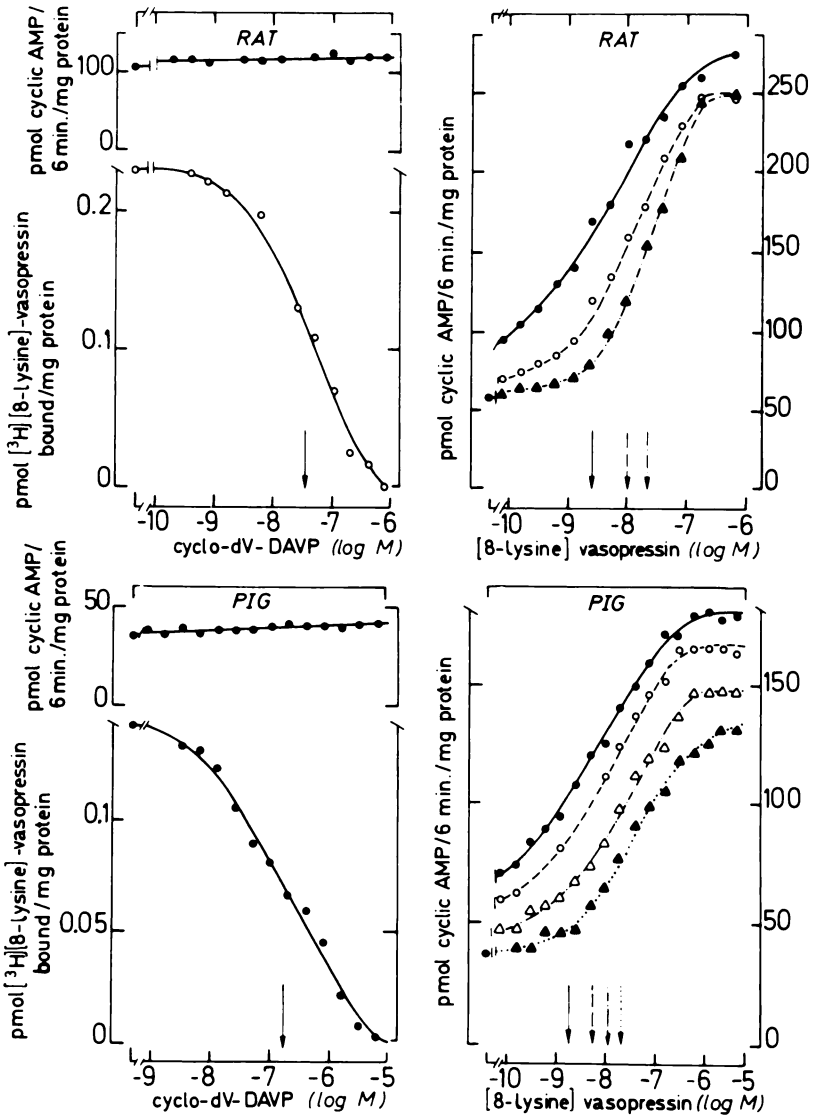

FIG. 3. *Inhibition of [8-lysine]-vasopressin-induced adenylate cyclase activation by [1(β-mercapto-β,β-cyclopentamethylene propionic acid), 4-valine, 8-D-arginine]-vasopressin on rat and pig system*

The upper panels illustrate the results obtained on the rat system and the lower panels, the porcine system. Due to a shortage of [8-arginine]-vasopressin, [8-lysine]-vasopressin was used as active peptide in this experiment. Left panel: see legend to Fig. 2. The K_{bind} values for cyclo-dV-DAVP were: 12 nM (rat) and 130 nM (pig). The concentrations of [^{3}H]lysine-vasopressin used were 7 nM (rat kidney membranes) and 5.3 nM (pig kidney membranes). The right panels show dose-dependent adenylate cyclase activation by [8-lysine]-vasopressin added alone (●) and in presence of cyclo-dV-DAVP. For the experiment with rat kidney membranes two concentrations of cyclo-dV-DAVP were used: 20 nM (○) and 100 nM (▲). The Hill coefficients of the activation curves were n = 0.56 (●), 0.69 (○) and 0.96 (▲). The inhibition constant K_i = 10 nM was calculated as indicated in the legend to Fig. 4. For the experiment with pig kidney membranes, 3 different concentrations of cyclo-dV-DAVP were used: 0.25 μM (○), 1.2 μM (△), and 4.8 μM (▲). The Hill coefficients of the activation curves were n = 0.46 (●), 0.49 (○), 0.53 (△) and 0.72 (▲) and the K_i value was 0.57 μM. Arrows on the left panels indicate I_{50} values. Arrows on the right panels indicate K_{act} values for lysine-vasopressin (solid lines), and A_{50} values: i.e., concentrations of lysine-vasopressin leading to half maximal activation in presence of cyclo-dV-DAVP (dotted lines).

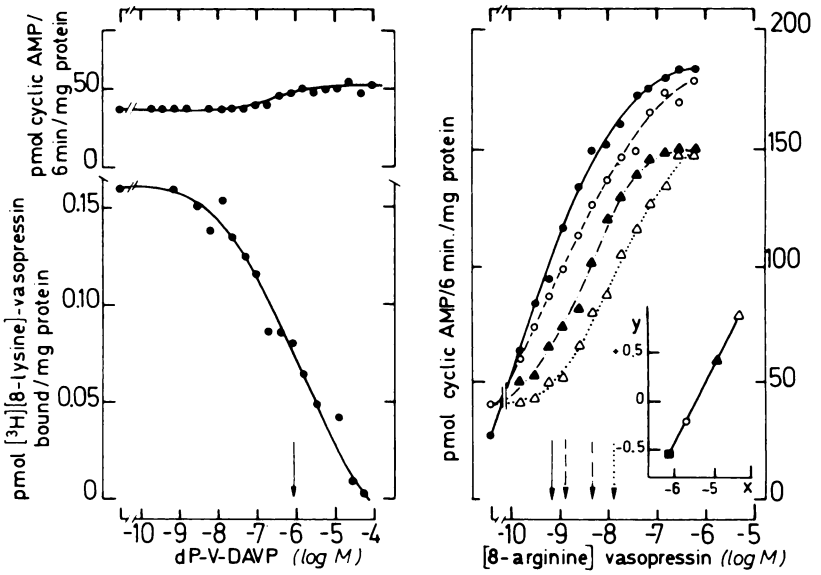

FIG. 4. *Inhibition of [8-arginine]-vasopressin-induced adenylate cyclase activation by [1-deaminopenicillamine, 4 valine, 8-D-arginine]-vasopressin in the pig system*

Left panel: The upper part shows adenylate cyclase activity measured in presence of the indicated amounts of dP-V-DAVP and the lower part shows the dose-dependent inhibition of [^{3}H]lysine-vasopressin (2.5 nM) binding by unlabeled dP-V-DAVP. The K_{bind} value for the peptide, calculated with the method indicated in the legend to Fig. 2, was 0.64 μM. Right panel: Dose-dependent adenylate cyclase activation by 8-arginine-vasopressin was determined under 5 experimental conditions: in absence of dP-V-DAVP (●) and presence of different concentrations of dP-V-DAVP, 0.67 μM (not shown), 2 μM (○), 12 μM (▲) and 50 μM (△). Arrows on the graph are K_{act} value for arginine-vasopressin and A_{50} values, i.e., concentrations of arginine-vasopressin leading to half maximal activation in presence of dP-V-DAVP. The Hill coefficient of the activation curve for arginine-vasopressin alone was 0.65, and the corresponding values measured in presence of dP-V-DAVP were 0.69 (■, 0.67 μM), 0.70 (○), 0.71 (▲) and 0.74 (△). The inserted figure shows the linear relationship between x = log (dP-V-DAVP)

$$\text{and} \qquad Y = \log \frac{A_{50}{}^{n_i} - K_{act}{}^{n}}{K_{act}{}^{n}}$$

The explanation appears in the text. The slope of the regression line was $m = 0.76$; the inhibition constant K_i deduced from the experimental curve was 4.3 μM.

imal activation in the presence of inhibitor):

$$\log (A_{50}{}^{n_i} - (K_{act})^n = m\log [I] - m\log (K_i)$$

As shown by fig. 4 this relation was satisfied for different concentrations of inhibitor. Therefore the constant K_i (which is independent of [I]) was considered as a satisfactory estimation of the inhibition constant.

Finally, when considering the entire series of analogues tested, there was a good correlation between the K_{bind} values for binding to renal membranes and the corresponding K_{act} or K_i values for adenylate cyclase response (Fig. 5).

DISCUSSION

Previous studies (4, 5) have shown that for a series of 30 structural analogues of oxytocin and vasopressin there was a good correlation between the apparent K_{bind} values for attachment to pig kidney membranes and the corresponding K_{act} values for adenylate cyclase activation. The present study confirms this conclusion for another series of arginine-vasopressin analogues. In addition, it shows that a similar correlation exists for the rat system. Such identical stereospecificities for hormonal binding and response strongly suggest that

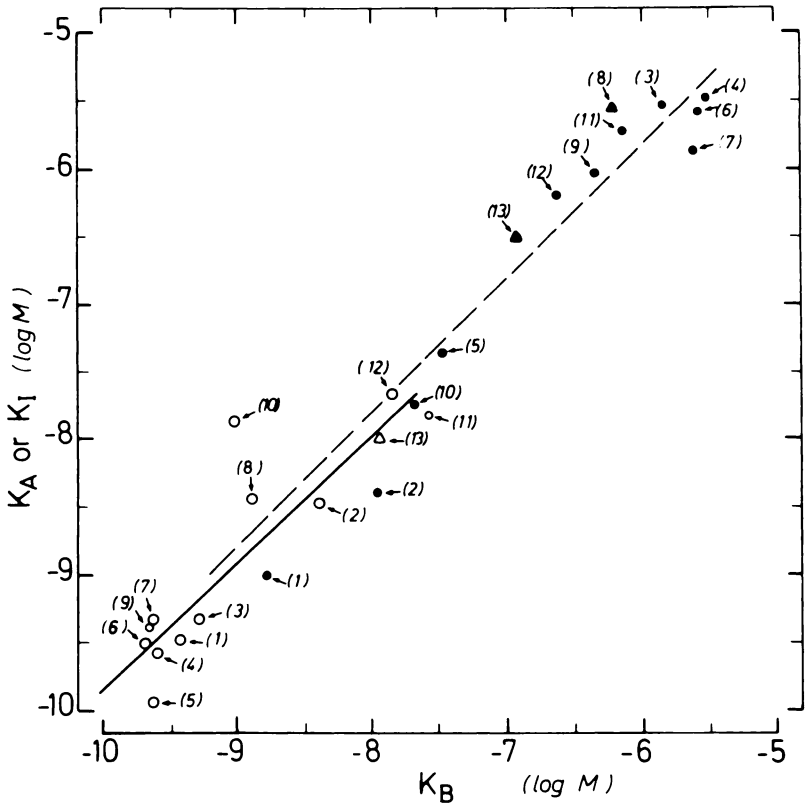

FIG. 5. *Correlation between (K_{bind}) and (K_{act}) or K_i values for [8-arginine]-vasopressin and analogues*
The figure was constructed from the data presented in Table 2. K_{act} or K_i was plotted as a function of the corresponding K_{bind} values. Unfilled symbols correspond to data obtained using the rat system, filled symbols correspond to those obtained in the pig system. The correlation coefficient and the equation of the regression line were $r = 0.88$, $p < 0.01$; $y = 0.92\ x - 0.65$, for the rat systems (solid line), and $r = 0.96$, $p < 0.01$, $y = 1.16\ x + 1.11$ for the pig system (dotted line). Key to symbols: (1), AVP; (2), [^{3}H]LVP; (3), DAVP; (4), d-DAVP; (5), V-AVP; (6), V-DAVP; (7), dV-DAVP; (8), dP-V-DAVP; (9), dT-DAVP; (10), Tyr (OMe)-AVP; (11), dP-Tyr (OMe)-AVP; (12), dP-AVP; (13), cyclo-dV-DAVP.

the detected binding sites are the specific receptors involved in adenylate cyclase activation. From the data reported in Table 2, it is apparent that all the analogues tested had a lower affinity for the pig renal receptor than arginine-vasopressin. All peptides containing D-arginine in place of L-arginine had markedly reduced affinities. Substitution of valine for glutamine or methylation of tyrosine in position 2 led to an about 20-fold reduction in affinity. Introduction of a deaminopenicillamine group in the first position reduced by about 100-fold the affinity for the receptor, and at the same time maximal adenylate cyclase activation was reduced as compared to arginine-vasopressin. Methylation of tyrosine dP-AVP led to a further reduction in the V_{max} value. Finally the two peptides that behaved like competitive inhibitors of lysine-vasopressin-induced adenylate cyclase activation were modified in position 1; they contained a valine residue in position 4 and D-arginine in position 8.

Marked differences between the rat and pig systems are apparent from the data reported in Table 2. In the rat system, substitution of D-arginine for L-arginine did not lead to a marked decrease in affinity. Introduction of a deaminopenicillamine group in position 1 increased markedly the V_{max} value; introduction of valine in position 4 and of D-arginine in position 8 reduced the V_{max} value; dP-V-DAVP, in

TABLE 3

Potential antidiuretic activities of vasopressin analogues

Compounds	$\frac{K_{act}AVP}{K_{act}\text{ analogue}}$ (A)	$\frac{V_{max}\text{ analogue}}{V_{max}\text{ AVP}}$ (B)	$\frac{T\text{ analogue}}{T\text{ AVP}}$ (C)[a]	Index of potential activity *in vivo* (A × B × C)	Antidiuretic activity (analogue/AVP)[b]
AVP	1.000	1.00	1.00	1.00	1.00
DAVP	0.729	0.96	1.31	0.92	0.77
V-DAVP	1.061	1.20	1.31	1.67	1.97
V-AVP	2.917	0.73	1.38	2.94	2.22
dT-DAVP	0.814	0.94	5.00	3.83	2.39
d-DAVP	1.250	0.88	3.00	3.30	2.88
dV-DAVP	0.778	1.23	4.54	4.34	3.70

[a] C was calculated from values reported by Sawyer *et al.* (14, 15, and unpublished data) for the duration of the antidiuretic action of arginine-vasopressin and analogues (5–25 mI.U./kg).

[b] Values calculated from data reported in Table 1, using a value of 332 I.U./mg for the antidiuretic activity of arginine-vasopressin.

which these three modifications were introduced, induced a maximal adenylate cyclase activation that was lower than that obtained with arginine-vasopressin. Only cyclo-dV-DAVP behaved like a competitive inhibitor. This peptide is the best known inhibitor of rat and pig adenylate cyclase activation by vasopressin.

For the analogues tested, large discrepancies exist between antidiuretic activities determined *in vivo* in the rat and the corresponding K_{bind} or K_{act} values determined *in vitro* (compare Tables 1 and 2). Several factors have to be considered when evaluating the antidiuretic potency of vasopressin and analogues when tested *in vivo*. Among these factors are distribution space and elimination rate from body fluids, affinity for the renal receptor, activity on the adenylate cyclase system, and the dependence of the final response on the level of intracellular cyclic AMP. Previous studies have indicated that the membrane preparation used for binding and adenylate cyclase assays did not inactivate arginine-vasopressin and several of its structural analogues (3–6). Therefore the data reported in the present study can be considered as correct estimations of maximal enzyme activations by the peptides tested and of their relative affinities. Potential antidiuretic activities of these peptides were calculated using the following relation:

$$P = \frac{V_{max}}{K_{act}} \times T$$

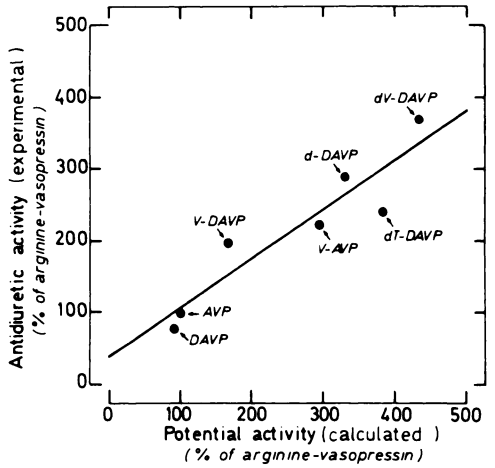

FIG. 6. *Correlation between antidiuretic activity determined* in vivo *and potential activity calculated from the data obtained* in vitro.

The figure was constructed from the data given in Table 3. All values are expressed as percentages of those corresponding to [8-arginine]-vasopressin. The correlation coefficient is $r = 0.92$, $p < 0.01$. The equation of the regression line is $y = 0.68\ x + 40$.

in which T was the duration of the antidiuretic effect. The values of T were taken from Sawyer *et al.* (14, 15) and unpublished data. The P values calculated are given in Table 3. As shown by Fig. 6, there was a satisfactory correlation between these two sets of values. Such an observation suggests that the adenylate cyclase system present in the acellular preparation used has the same stereospecificity as that of the adenylate cyclase functioning *in vivo*.

The K_{bind} value obtained for arginine-vasopressin is much higher than concentrations of circulating ADH, even under experimental conditions in which hormone secretion rate is maximally stimulated. This might indicate that only a small fraction of the cyclic AMP that can be produced by maximally stimulated adenylate cyclase is sufficient to induce a maximal antidiuretic response. One can estimate that for a blood ADH concentration of 0.01 nM the magnitude of adenylate cyclase activation is only 5% of the maximal activation. From measurement of K_{bind} for arginine-vasopressin (0.4 nM) one can calculate that this 5% adenylate cyclase activation is induced by occupation of 2.5% of the total number of receptors. In other words, a weak agonist with a V_{max} value that is higher or equal to 5% of the V_{max} value of arginine-vasopressin can be expected to be able to induce a full antidiuretic response *in vivo*. This might explain why cyclo-dV-DAVP, with a V_{max} value that is 7% of the V_{max} value of arginine-vasopressin, has a very weak but significant antidiuretic activity while its behavior is one of an almost purely competitive inhibitor on the *in vitro* system.

REFERENCES

1. Douša, T., Hechter, O., Walter, R. & Schwartz, I. L. (1970) *Science,* **167,** 1134–1135.
2. Douša, T., Hechter, O., Schwartz, I. L. & Walter, R. (1971) *Proc. Natl. Acad. Sci. U.S.A.,* **68,** 1693–1697.
3. Bockaert, J., Roy, C., Rajerison, R. M. & Jard, S. (1973) *J. Biol. Chem.,* **248,** 5922–5931.
4. Roy, C., Barth, T. & Jard, S. (1975) *J. Biol. Chem.,* **250,** 3149–3156.
5. Roy, C., Barth, T. & Jard, S. (1975) *J. Biol. Chem.,* **250,** 3157–3164.
6. Barth, T., Roy, C., Rajerison, R. M. & Jard, S. (1975) *Mol. Cell. Endocrinol.,* **2,** 69–80.
7. Barth, T., Rajerison, R. M., Roy, C. & Jard, S. (1975) *Mol. Cell. Endocrinol.,* **2,** 81–90.
8. Rajerison, R. M., Marchetti, J., Roy, C., Bockaert, J. & Jard, S. (1974) J. Biol. Chem., **249,** 6390–6400.
9. Rajerison, R. M., Butlen, D. & Jard, S. (1976) *Mol. Cell. Endocrinol.,* **4,** 271–285.
10. Stürmer, E. (1968) in *Handbüch der Experimentellen Pharmakologie,* Vol. XXIII, (Berde, B., ed.), pp. 130–189, Springer-Verlag, Berlin.
11. Barth, T. & Kluh, I. (1974) *Collect. Czech. Chem. Çommun.,* **39,** 279–282.
12. Barth, T., Krejči, I., Kupková, B. & Jošt, K. (1973) *Eur. J. Pharmacol.,* **24,** 183–188.
13. Manning, M., Balaspiri, L., Acosta, M. & Sawyer, W. H. (1973) *J. Med. Chem.,* **16,** 975–978.
14. Sawyer, W. H., Acosta, M., Balaspiri, L., Judd, J. & Manning, M. (1974) *Endocrinology,* **94,** 1106–1115.
15. Sawyer, W. H. Acosta, M. & Manning, M. (1974) *Endocrinology* **95,** 140–149.
16. Manning, M., Balaspiri, L., Judd, J., Acosta, M. & Sawyer, W. H. (1974) *FEBS Lett.,* **44,** 229–232.
17. Manning, M., Blaspiri, L., Moehring, J., Haldar, J. & Sawyer, W. H. (1976) *J. Med. Chem.,* **19,** 842–845.
18. Manning, M., Lowbridge, J., Stier, C. T., Jr., Halder, J. & Sawyer, W. H. (1977) *J. Med. Chem.,* **20,** 1228–1230.
19. Lowbridge, J., Manning, M., Haldar, J. & Sawyer, W. H. (1978) *J. Med. Chem.,* **21,** 313–315.
20. Prusík, Z., Sedláková, S. E. & Barth, T. (1972) *Hoppe-Seyler's Z Physiol. Chem.,* **353,** 1837–1840.
21. Pradelles, P., Morgat, J. L., Fromageot, P., Camier, M., Bonne, D. Cohen, P., Bochaert, J. & Jard, S. (1972) *FEBS Lett.,* **26,** 189–192.
22. Salomon, Y., Londos, C. & Rodbell, M. (1974) *Anal. Biochem.,* **58,** 541–548.
23. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.,* **193,** 265–275.