Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 2907–2911, May 1984
Neurobiology

Properties of rat anterior pituitary vasopressin receptors: Relation to adenylate cyclase and the effect of corticotropin-releasing factor

(adrenocorticotropic hormone/angiotensin/adenylate cyclase inhibition/vasopressin analogues)

R. C. GAILLARD*, P. SCHOENENBERG*, C. A. FAVROD-COUNE*, A. F. MULLER*, J. MARIE†, J. BOCKAERT†, AND S. JARD†

*Clinique Médicale, Hôpital Cantonal Universitaire, 1211 Geneva 4, Switzerland; and †Centre National de la Recherche Scientifique, Institut National de la Santé et de la Recherche Médicale de Pharmacologie-Endocrinologie, BP 5055, 34033 Montpellier, France

Communicated by S. M. McCann, December 1, 1983

ABSTRACT **Crude plasma membrane fractions were prepared from female Wistar rat anterior pituitaries. These fractions contained a single population of specific ^{3}H-labeled [8-lysine]vasopressin ([^{3}H]vasopressin) binding sites with a dissociation of constant (K_d) of 8 ± 2 × 10^{-9} M and maximal binding capacity of 244 ± 45 fmol/mg of protein. The K_d values for a series of vasopressin structural analogues with selective vasopressor or antidiuretic activities were determined together with the corresponding corticotropin-releasing activities (isolated perfused pituitary cells were used). A good correspondence was found between the two sets of values, suggesting that the detected vasopressin binding sites are the receptors involved in vasopressin-induced corticotropin release. The order of potency of these analogues for the binding to hypophysial receptors was similar to that found for the binding to the receptors involved in the vasopressor response. Corticotropin-releasing factor and angiotensin did not affect vasopressin binding to pituitary membranes. Median eminence extracts inhibited [^{3}H]vasopressin binding with an efficiency very close to that expected from their vasopressin content. Corticotropin-releasing factor activated, and angiotensin inhibited, the adenylate cyclase activity of pituitary membranes. Under the same experimental conditions, vasopressin did not influence adenylate cyclase activity nor did it affect the corticotropin-releasing factor-induced activation. These data support the view that vasopressin is one component of the multifactorial regulation of corticotropin release and that it acts through a cAMP-independent pathway. The potentiation by vasopressin of corticotropin-releasing factor-induced cAMP accumulation in intact cells very likely proceeds through indirect mechanisms, which are not expressed in broken cell preparations.**

Despite abundant evidence (1–6) that the 41-residue peptide recently isolated from ovine hypothalami (7) is a physiological corticotropin-releasing factor (CRF), the hypothalamic factor responsible for the integrated release of corticotropin (ACTH) seems not to be a single peptide but a multifactorial complex (8, 9). Vasopressin has been proposed as a constituent of this complex (9, 10). There are several pieces of evidence in favor of this concept: the synergism observed between ovine CRF and vasopressin (10, 11); the coexistence of immunoreactive neurophysin/vasopressin and CRF in nerve terminals of the zona externa of the median eminence (12) and in cells of the paraventricular nucleus (13); and finally high levels of vasopressin in portal blood (14). Further support for the role of vasopressin in the CRF complex includes the potentiation of the Brattleboro rat CRF activity by addition of synthetic vasopressin (15) and recent chromatographic investigations (9, 15).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Nevertheless, the properties of vasopressin receptors that confer CRF activity are not well defined. The relationship between CRF and pressor or antidiuretic activity of various vasopressin analogues is in dispute (16–20). Recently Aizawa *et al.* found that CRF and pressor activities of several vasopressin analogues were strongly correlated, whereas CRF and antidiuretic activities were not (21). These data were obtained from *in vivo* experiments; therefore, it cannot be ascertained that the CRF effects of the analogues were due to a direct effect on the pituitary. This CRF effect may indeed be due to the vasoconstrictor action of these drugs, which could indirectly induce corticotropin secretion through some intermediary mechanism.

Although a role for vasopressin as a physiological CRF is now being considered, the characterization of pituitary vasopressin receptors is still missing. This work was undertaken to clarify the relationship between CRF and other pharmacological activities of vasopressin and several analogues by investigating the binding-activation properties of vasopressin receptors at the pituitary level.

MATERIALS AND METHODS

Materials. ^{3}H-labeled [8-lysine]vasopressin ([^{3}H]vasopressin, 8 Ci/mM; 1 Ci = 37 GBq) was prepared as described (22). [^{3}H]Vasopressin was purified by affinity chromatography using neurophysin-Sepharose columns. The biological activities of the labeled peptide were found indistinguishable from those of the starting materials (synthetic [8-lysine]vasopressin from UCB Bau Products SA, Brussels, Belgium). The following peptides were used: [8-arginine]vasopressin ([Arg^8]VP), [8-lysine]vasopressin ([Lys^8]VP), oxytocin, [1-(L-2-hydroxy-3-mercaptopropanoic acid), 8-arginine]vasopressin designated [(OH)^1, Arg^8]VP, [1-(L-2-hydroxy-3-mercaptopropanoic acid), 4-valine, 8-D-arginine]vasopressin designated [(OH)^1, Val^4, D-Arg^8]VP, [4-valine-8-D-arginine]vasopressin ([Val^4, D-Arg^8]VP), 1-deamino[8-D-arginine]vasopressin (1-deamino[D-Arg^8]VP), and [2-phenylalanine, 8-ornithine]vasotocin ([Phe^2, Orn^8]VT).

[1-asparagine, 5-valine]Angiotensin II ([Asn^1, Val^5]AII) was purchased from UCB. CRF was a generous gift from N. Ling. [γ^{32}P]ATP was purchased from New England Nuclear; trypsin and lima bean trypsin inhibitor, from Worthington; Earle's balanced salt solution (EBSS), from GIBCO; Trasy-

Abbreviations: [Arg^8]VP, [8-arginine]vasopressin; [Lys^8]VP, unlabeled [8-lysine]vasopressin; [^{3}H]vasopressin, ^{3}H-labeled [8-lysine]-vasopressin; (OH), $HSCH_2CH(OH)COOH$ (replacement for 1-cysteine of vasopressin); [(OH)^1, Arg^8]VP, [1-(L-2-hydroxy-3-mercaptopropanoic acid), 8-arginine]vasopressin; [(OH)^1, Val^4, D-Arg^8]VP, [1-(L-2-hydroxy-3-mercaptopropanoic acid), 4-valine, 8-D-arginine]-vasopressin; [Val^4, D-Arg^8]VP, [4-valine, 8-D-arginine]vasopressin; 1-deamino[D-Arg^8]VP, 1-deamino[8-D-arginine]vasopressin; [Phe^2, Orn^8]VT, [2-phenylalanine, 8-ornithine]vasotocin; [Asn^1, Val^5]AII, [1-asparagine, 5-valine]angiotensin II; CRF, corticotropin-releasing factor.

lol, from Bayer (Haywards Heath, Sussex, England). All other chemicals were A grade.

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°C 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. For the adenylate cyclase assay, six adenohypophyses were homogenized in 1 ml of TES buffer (1 mM Tris maleate, pH 7.2/1 mM EGTA/10% sucrose). The homogenate was filtered through a silk screen (0.2-mm pore size) and diluted with 2 ml of TES buffer without sucrose. The membrane suspension was centrifuged at 10,000 × *g* for 15 min, and the pellet resuspended in 1.5 ml of TES buffer. A total of 11 membrane preparations was used.

The isolated rat anterior pituitary cell column used for CRF bioassays was prepared as described by Gillies and Lowry (23). Briefly, the cells from five adenohypophyses were dispersed by mechanical agitation in 0.25% trypsin solution, mixed with 0.5 g of preswollen Bio-Gel P-2 (200–400 mesh), and packed into a 2-ml plastic column (0.9 × 3 cm). 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).

Crude stalk median eminence extracts were prepared as follows. The hypothalamic–hypophysial stalk, with a small area of median eminence approximately 3 mm in diameter surrounding it (3–4 mg wet weight) was removed at the same time as the pituitaries. The median eminence fragments were homogenized in 0.01 M HCl containing ascorbic acid (1 mg/ml) at a concentration of 1 median eminence extract per ml. After neutralization with 1.1 M $NaHCO_3$ (10 μl/ml) and addition of NaCl (9 mg/ml) and Trasylol (250 units/ml), the extract was centrifuged (20 min at 3,000 × *g*), and the supernatant was frozen until used in binding assays.

[^{3}H]Vasopressin Binding Assay. 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), and [^{3}H]vasopressin (1.25–40 nM) (total volume 200 μl). Incubation was performed at 30 or 37°C for 15 min. The reaction was initiated by the addition of membranes and stopped by addition of 4 ml of cold 10 mM Tris·HCl, pH 7.4/1 mM $MgCl_2$, 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 [Lys^8]VP. Radioactivity measurements were performed by liquid scintillation spectrometry. Under standard incubation conditions no marked degradation of [^{3}H]vasopressin occurred (less than 5% within 15 min and 15% in 60 min). [^{3}H]Vasopressin degradation was checked by HPLC. [^{3}H]Vasopressin specific binding increased linearly with membrane concentration up to 200 μg of protein. For 5 nM [^{3}H]vasopressin (a concentration close to the apparent dissociation constant, K_d) nonspecific binding represented 13 ± 6% (11 determinations) of total binding. All determinations were performed in triplicate.

Adenylate Cyclase Assay. Membranes were incubated for 15 or 30 min at 30°C in 50 mM Tris maleate (pH 7.2) containing $MgSO_4$ (1–2.5 mM), cAMP (1 mM), GTP (10 μM), theophylline (10 mM), creatine kinase (0.1 mg/ml), creatine phosphate (5 mM), ATP (0.15 mM), [^{3}H]cAMP (0.02 μCi/ml), and [γ^{32}P]ATP (20–40 μCi/ml). The total incubation volume was 50 μl. The reaction was initiated by the addition of membranes (10 μl). Labeled cAMP formed was purified as described (24). All determinations were performed in triplicate.

Bioassay for CRF Activity. Cells were stimulated with 3-min pulses of the test substance at 14-min intervals. The column effluent was collected as 2-min fractions, frozen, and stored at −20°C. The corticotropin content in the fractions was determined by a direct RIA (25). The results were expressed as total corticotropin released by a pulse of vasopressin or one of its analogues in excess of background secretion. Each measurement of the CRF activity of a given vasopressin analogue was calculated from the determinations of six experiments, each involving three dose–response curves for that analogue and 2–3 dose–response curves for [Arg^8]VP used as a standard. Cells were matched with five [Arg^8]VP concentrations ranging between 0.3 and 30 nM and given in random order. The mean basal secretion of corticotropin was 117 ± 16 pg. The total amount of corticotropin released in response to a 3-min pulse of 3 nM [Arg^8]VP per ml—a dose close to the ED_{50} of 4.3 ± 0.6 nM—was 2.5 ± 0.17 ng (mean ± SEM).

RESULTS AND DISCUSSION

[^{3}H]Vasopressin binding to adenohypophysial membranes was time- and temperature-dependent. It was reversible upon addition of unlabeled [Lys^8]VP and saturable (Fig. 1).

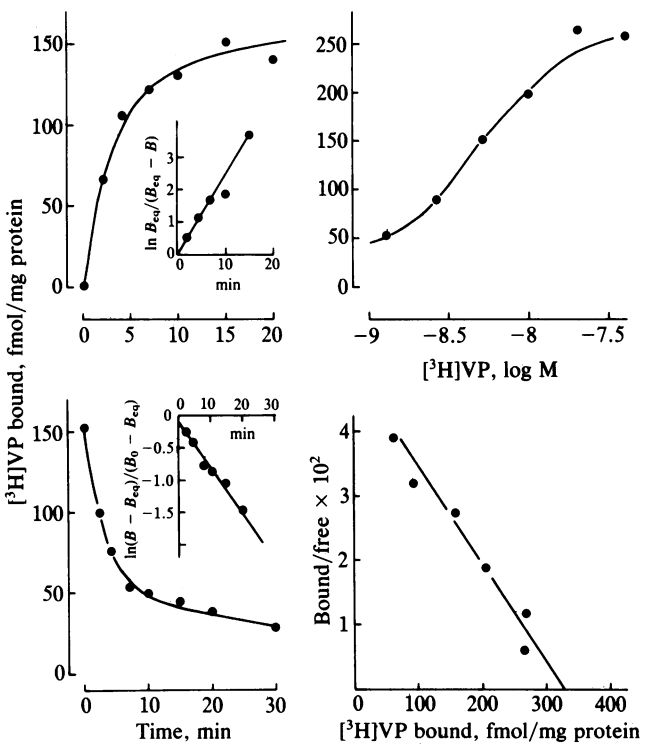

FIG. 1. Kinetics of [^{3}H]vasopressin ([^{3}H]VP) binding to rat anterior pituitary membranes. (*Upper Left*) Time course of specific [^{3}H]vasopressin binding. The concentration of [^{3}H]vasopressin added at 0 time was 5 nM. (*Insert*) Logarithmic transform of the association curve. (*Lower Left*) Time course of hormone–receptor dissociation. Membranes were first incubated for 15 min in the presence of 5 nM [^{3}H]vasopressin. Dissociation was induced by addition of unlabeled vasopressin. (*Insert*) Logarithmic transform of the dissociation curve. The value of B_{eq} leading to the best linear plot represents 20% of the initial binding (B_0). The computed rate constant for the formation (k_1) and dissociation (k_{-1}) of the hormone–receptor complexes were $k_1 = 2.1 \times 10^7$ M^{-1}·min^{-1}; $k_{-1} = 0.17$ min^{-1}. (*Upper Right*) Dose-dependent [^{3}H]vasopressin binding at equilibrium (15-min incubation period). (*Lower Right*) Scatchard plot of the dose binding curve from which the K_d and maximal binding capacity (B_{max}) were deduced. $K_d = 5.6 \times 10^{-9}$ M; $B_{max} = 330$ fmol/mg of protein.

Both the association and dissociation time courses were monoexponential processes as expected from a pseudo-first-order reaction. The dose-dependency for [^{3}H]vasopressin binding at equilibrium did not reveal a marked heterogeneity in the population of vasopressin binding sites, as indicated by a linear Scatchard plot.

There was a fairly good correspondence between the K_d deduced from the association (k_1) and dissociation (k_{-1}) rate constants ($k_1 = 2.1 \times 10^7$ M^{-1}·min^{-1}; $k_{-1} = 0.17$ min^{-1}; $K_d = 8.1 \times 10^{-9}$ M) and that derived from dose-dependent binding at equilibrium ($K_d = 5.6 \times 10^{-9}$ M). A mean K_d value of 8 ± 2 (SD) × 10^{-9} M, deduced from seven independent determinations, was close to those determined for vasopressin receptors in rat liver membranes (26) and rat aortic myocytes (27). It was higher than that of adenylate cyclase-coupled vasopressin receptors from rat kidney membranes (28). A mean maximal binding capacity of 244 ± 45 ($n = 7$) fmol/mg of protein was found. At the pituitary level, beside its CRF activity, vasopressin has been reported to release thyroid-stimulating hormone (29). Therefore, if one assumes that vasopressin binding sites are located on both corticotrophic and thyrotrophic cells, each representing about 4% of the total population of pituitary cells [2.5 × 10^6 cells per hypophysis (30)], the latter value would indicate that the maximal vasopressin binding capacity of corticotrophs is 60 fmol per 10^6 cells, a figure that is close to that found for rat hepatocytes, 320 fmol per 10^6 cells (26).

The relative affinities of a series of vasopressin analogues for the detected [^{3}H]vasopressin sites were determined from competition experiments similar to those shown in Fig. 2. The results obtained are summarized in Table 1 together with the results of the determination of the CRF-like activities of these analogues. When comparing these two sets of data, a good correlation between the relative affinities of the tested analogues for pituitary membranes and the corresponding relative potencies in inducing corticotropin release could be demonstrated (see Table 1). In addition, the K_d value for [Arg^8]VP binding (4 nM) is close to the apparent K_a for [Arg^8]VP-induced corticotropin release (4.3 ± 0.6 nM). These two observations strongly suggest that the detected vasopressin binding sites are the physiological receptors involved in the CRF-like activity of vasopressin. In most vasopressin target cells so far studied, the apparent K_a for the biological response was found to be much lower than the K_d. This observation was accounted for by the existence of a large receptor reserve (see, for instance, refs. 26 and 27). The present results would indicate that such a large receptor reserve does not exist in the case of pituitary cells. It is noteworthy that pituitary cells are exposed to vasopressin concentrations that are much higher than the systemic vasopressin concentration. Vasopressin concentrations as high as 13 nM have been determined in the hypophysial portal blood in the monkey (14).

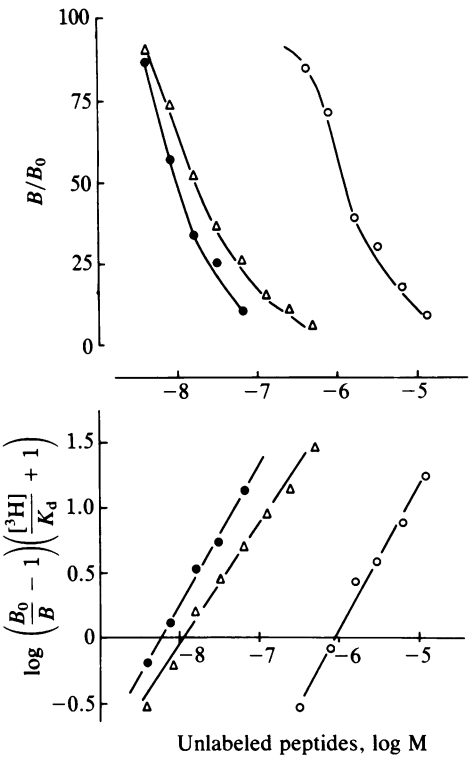

FIG. 2. Inhibition of [^{3}H]vasopressin binding by unlabeled vasopressin and vasopressin analogues. (*Upper*) Membranes were incubated in the presence of 5 nM [^{3}H]vasopressin and the indicated amounts of unlabeled peptides. B_0, specific binding in the absence of unlabeled peptide; B, specific binding in the presence of unlabeled peptide; ●, [Arg^8]VP; △, [Phe^2, Orn^8]VT; ○, 1-deamino[D-Arg^8]VP. (*Lower*) Logarithmic transform of the displacement curves. [^{3}H], concentration of [^{3}H]vasopressin; K_d, dissociation constant for [^{3}H]vasopressin binding determined in the course of the same experiment. Note that the slopes of the regression lines are close to unity. The dissociation constant for binding of the unlabeled analogues (identified as in *Upper*) was deduced from the x intercepts of the curves.

Data shown in Table 1 also indicate that the order of potency found for the binding of the tested analogues to the hypophysial membranes is similar to that found in the rat vasopressor assay. It is markedly different from that found in the rat antidiuretic assay. Thus, analogues exhibiting a high vasopressor/antidiuretic activity ratio such as [(OH)^1, Arg^8]VP and [Phe^2, Orn^8]VT are the most potent in inhibiting [^{3}H]vasopressin binding. The L/D-arginine substitution

Table 1. Relative affinities of vasopressin analogues for rat anterior pituitary receptors: Relation to corticotropin-releasing, vasopressor, and antidiuretic activities

Peptide	Affinity for anterior pituitary receptor	Biological activities*		
		Corticotropin release	Vasopressor	Antidiuretic
[Arg^8]VP	100	100	100	100
[Lys^8]VP	68	56 ± 4	73	88
Oxytocin	1.1	4.6 ± 0.7	1.1	1.3
[(OH)^1, Arg^8]VP	113	153 ± 15	149	146
[Phe^2, Orn^8]VT	70	74 ± 7	33	0.17
[Val^4, D-Arg^8]VP	ND	2.4 ± 0.3	0.01	202
[(OH)^1, Val^4, D-Arg^8]VP	1.3	3.6 ± 0.5	0.01	276
1-deamino[D-Arg^8]VP	0.9	3.0 ± 0.4	0.11	371

All values are relative values with [Arg^8]VP as a standard. ND, not determined.
*Values used for the calculation of relative vasopressor and antidiuretic activities were taken from ref. 31. This article also contains references for the synthesis of the tested analogues.

in position 8, and introduction of a valine residue in position 4, which were found to reduce markedly the vasopressor or glycogenolytic activities but to preserve the antidiuretic activity, have a low affinity for vasopressin receptors from rat pituitary {compare 1-deamino[D-Arg^8]VP to [Arg^8]VP and [(OH)^1, Val^4, D-Arg^8]VP to [(OH)^1, Arg^8]VP}. The detected vasopressin binding sites are specific for vasopressin and vasopressin-related peptides. CRF at concentrations up to 25 nM [i.e., 10 times the equilibrium dissociation constant for CRF binding to its specific receptors (32)] did not inhibit [^{3}H]vasopressin binding (Fig. 3). Dose-dependent [^{3}H]vasopressin binding was unaffected by the presence of 25 nM CRF in the incubation medium. Similarly, [Asn^1, Val^5]AII did not inhibit [^{3}H]vasopressin binding. However, median eminence extracts inhibited [^{3}H]vasopressin binding in a dose-dependent manner. The inhibitory potency of the extract expressed in terms of equipotent amounts of [Arg^8]VP was 7 ng per median eminence. The latter value compares very well with the effective [Arg^8]VP content of rat median eminence, according to Gillies *et al.* (33). Therefore, it can be concluded that none of the main factors involved in the regulation of corticotropin release interferes with vasopressin binding.

Fig. 4 summarizes the results derived from adenylate cyclase activity determinations. These results indicated that: (*i*) CRF stimulated pituitary adenylate cyclase activity in a dose-dependent manner; (*ii*) in line with recent results obtained on several [Asn^1, Val^5]AII-responsive tissues (34, 35), [Asn^1, Val^5]AII inhibited enzyme activity with an apparent K_i of 1×10^{-9} M close to its K_d value for binding of 4×10^{-9} M (36); (*iii*) under experimental conditions where both activation by CRF and inhibition by [Asn^1, Val^5]AII of adenylate cyclase activity could be demonstrated, vasopressin (10 nM to 10 μM) was ineffective; (*iv*) vasopressin (10 μM) did not affect dose-dependent activation by CRF; and (*v*) inhibition by [Asn^1, Val^5]AII (10 μM) could be expressed in the presence of increasing amounts of CRF. Therefore, it appears very likely that vasopressin receptors in rat pituitary are not functionally coupled to adenylate cyclase as already demonstrated for vascular and hepatic receptors (see for instance refs. 26 and 27). Data shown in Fig. 5 provide one indirect argument favoring this conclusion. [^{3}H]Vasopressin binding to pituitary membranes was, as already demonstrated for hepatic vasopressin receptors (26), inhibited by GTP and ATP in a dose-dependent manner. However, this effect which occurred in 0.1 mM range, is markedly different from the well-documented GTP effect on adenylate cyclase-coupled receptors (37). Fig. 5 also shows that vasopressin binding to anterior pituitary receptors exhibits an almost absolute requirement for magnesium ions. Our observation that vasopressin did not alter the adenylate cyclase activity of pituitary membranes confirms the report by Giguere *et al.* (38) that vasopressin alone does not modify intracellular cAMP content of isolated pituitary cells. However, these authors reported that vasopressin potentiated the CRF-induced cAMP accumulation in intact cells. Our results suggest that the potentiation of CRF action by vasopressin involves indirect mechanisms that cannot be expressed in a broken-cell preparation.

Altogether the above results strengthen the view that vasopressin is one component of a multifactorial regulation of corticotropin release. Besides CRF and vasopressin, several substances have been suggested as putative CRFs, including catecholamines, vasoactive intestinal peptide, and [Asn^1, Val^5]AII (6, 7, 10, 39, 40). The multimolecular nature of the hypothalamic factor responsible for corticotropin release is an attractive hypothesis because of the variety of stress situations. A CRF complex would provide a highly sensitive mechanism regulating very finely the stress hormone in response to a whole variety of endogenous and exogenous stimuli. Therefore, the various factors would have specific roles in various stress situations, and vasopressin may not play a role in all of them (41). This may explain some of the controversies about the physiological involvement of vasopressin in corticotropin release.

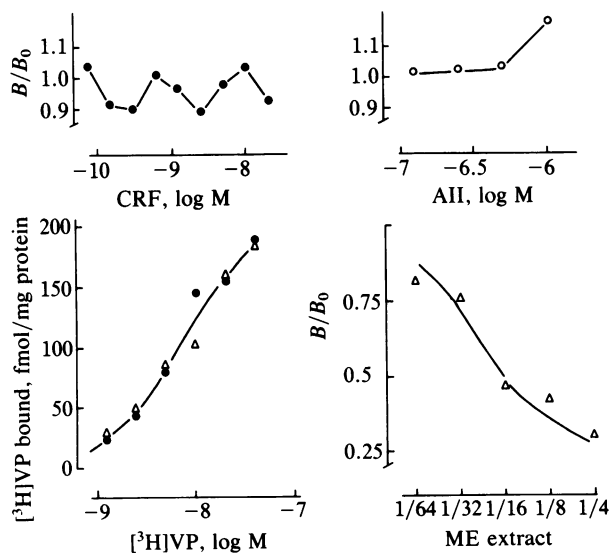

FIG. 3. Effects of CRF, [Asn^1, Val^5]AII (AII) and median eminence (ME) extract on [^{3}H]vasopressin binding to rat anterior pituitary membranes. Specific [^{3}H]vasopressin binding (5 nM) was determined in the absence (B_0) and the presence (B) of the indicated amounts of CRF (*Upper Left*), [Asn^1, Val^5]AII (*Upper Right*), or median eminence (ME) extract (*Lower Right*). In the latter case, the concentrations are expressed as dilution factors of the initial extract (see *Methods*). (*Lower Left*) Dose-dependent [^{3}H]vasopressin binding determined in the presence (△) and absence (●) of CRF (25 nM).

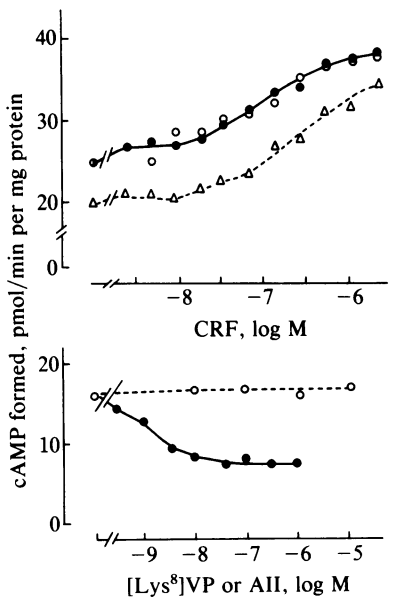

FIG. 4. Effects of [Lys^8]VP, CRF, and [Asn^1, Val^5]AII on rat anterior pituitary adenylate cyclase. Each point is the mean from triplicate incubations in one of three similar experiments. (*Upper*) Adenylate cyclase activity was determined in the presence of the indicated amounts of CRF added either alone (●) or in the presence of 100 nM [Lys^8]VP (○) or 100 nM [Asn^1, Val ^{5}]AII (△). The incubation medium contained 2.5 mM Mg^{2+}. (*Lower*) Adenylate activity was determined in the presence of the indicated amounts of [Lys^8]VP (○) or [Asn^1, Val^5]AII (AII) (●). The Mg^{2+} concentration was 1 mM. The reduction in Mg^{2+} as compared to the experiment shown in *Upper* is responsible for both a reduction in basal activity and enhancement of [Asn^1, Val^5]AII-induced inhibition.

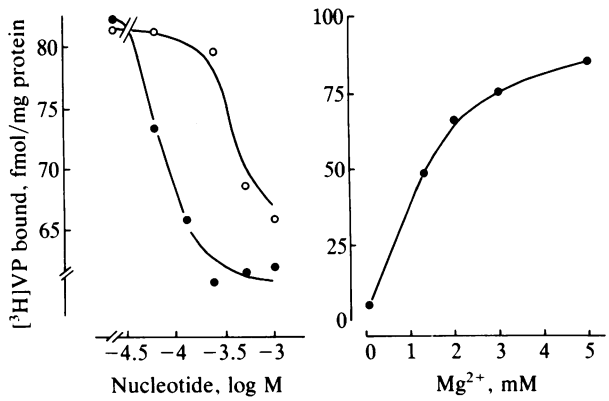

FIG. 5. Effects of ATP, GTP, and Mg^{2+} on [^{3}H]vasopressin binding to rat anterior pituitary receptors. [^{3}H]Vasopressin (5 nM) binding was determined in the presence of the indicated amounts of GTP (●) or ATP (○) (*Left*) or Mg^{2+} (*Right*). In the experiment where the nucleotide effect was tested, the incubation medium contained a triphosphonucleotide-regenerating system identical to that used for adenylate cyclase assay experiments.

The authors are indebted to Prof. M. Manning for providing the vasopressin structural analogues used in this study; Prof. L. H. Rees for the gift of corticotropin antibodies; Prof. N. Ling for the gift of CRF; Dr. J. L. Morgat for the preparation of [^{3}H]lysine vasopressin; the Mrs. R. Brögger, C. Lombard, M. C. Schupbach, and M. Sebben for skillful assistance; and Mrs. C. Todisco for the preparation of the manuscript. This work was supported by Centre National de la Recherche Scientifique, Institut National de la Santé et de la Recherche Médicale, Fondation pour la Recherche Médicale, and the Bickel-Birkigt, Maus-Nordmann, and Montus Foundations.

1. Bloom, F. E., Battenberg, E. L. F., Rivier, J. & Vale, W. (1982) *Regul. Peptides* **4**, 43–48.
2. Gibbs, D. M. & Vale, W. (1982) *Endocrinology* **111**, 1418–1420.
3. Grossman, A., Nieuwenhuyzen Kruseman, A. C., Perry, L., Tomlin, S., Schally, A. V., Coy, D. H., Comaru-Schally, A. M., Rees, L. & Besser, G. M. (1982) *Lancet* **i**, 921–922.
4. Moldow, R. L. & Fischman, A. J. (1982) *Peptides* **1**, 143–147.
5. Moldow, R. L. & Fischman, A. J. (1982) *Peptides* **3**, 837–840.
6. Rivier, C., Brownstein, M., Spiess, J., Rivier, J. & Vale, W. (1982) *Endocrinology* **110**, 272–278.
7. Vale, W., Spiess, J., Rivier, C. & Rivier, J. (1981) *Science* **213**, 1394–1397.
8. Pearlmutter, A. F., Rapino, E. & Saffran, M. (1975) *Endocrinology* **97**, 1336–1339.
9. Gillies, G. & Lowry, P. (1979) *Nature (London)* **278**, 463–464.
10. Gillies, G. E., Linton, E. A. & Lowry, P. J. (1982) *Nature (London)* **299**, 355–356.
11. Turkelson, C. M., Thomas, C. R., Arimura, A., Chang, D., Chang, D. K. & Shimizu, M. (1982) *Peptides* **1**, 111–113.
12. Tramu, G. & Pilley, A. (1982) *C.R. Acad. Sci. (Paris)* **295**, 107–114.
13. Roth, K. A., Weber, E. & Barchas, J. D. (1982) *Life Sci.* **31**, 1857–1860.
14. Zimmermann, E. A., Carmel, P. W., Husain, M. R., Ferin, M., Tannenbaum, M., Frantz, A. G. & Robinson, A. G. (1973) *Science* **182**, 925–927.
15. Gillies, G. & Lowry, P. J. (1980) *J. Endocrinol.* **84**, 65–73.
16. Pearlmutter, A. F., Rappino, E. & Saffran, M. (1974) *Neuroendocrinology* **15**, 106–119.
17. Portanova, R. & Sayers, G. (1973) *Proc. Soc. Exp. Biol. Med.* **143**, 661–666.
18. Arimura, A., Schally, V. A. & Bowers, C. Y. (1969) *Endocrinology* **84**, 579–583.
19. Andersson, K. E., Arnes, B., Hedner, P. & Mulder, J. L. (1972) *Acta Endocrinol. (Copenhagen)* **69**, 640–648.
20. Clayton, G. W., Librik, L., Gardner, R. L. & Guillemin, R. (1963) *J. Clin. Endocrinol.* **23**, 975–980.
21. Aizawa, T., Yasuda, N., Greer, M. A. & Sawyer, W. H. (1982) *Endocrinology* **110**, 98–103.
22. Pradelles, P., Morgat, J. L., Fromageot, P., Canier, M., Bonne, D., Cohen, P. & Bockaert, J. (1972) *FEBS Lett.* **26**, 189–192.
23. Gillies, G. & Lowry, P. J. (1978) *Endocrinology* **103**, 521–527.
24. Lucas, M. & Bockaert, J. (1977) *Mol. Pharmacol.* **13**, 314–329.
25. Rees, L. H., Cook, D. M., Kendall, J. W., Allen, O. F., Kramer, R. M., Ratcliffe, J. G. & Knight, R. A. (1971) *Endocrinology* **89**, 254–261.
26. Cantau, B., Keppens, S., De Wulf, H. & Jard, S. (1980) *J. Recep. Res.* **1**, 137–168.
27. Penit, J., Faure, M. & Jard, S. (1983) *Am. J. Physiol.* **244**, E72–E82.
28. Butlen, E., Guillon, G., Rajerisson, R. M., Jard, S., Sawyer, W. H. & Manning, M. (1978) *Mol. Pharmacol.* **14**, 1006–1007.
29. Lumpkin, M. D., Samson, W. K. & McCann, S. M. (1983) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **42**, 973 (abstr.).
30. Baker, B. L. (1974) in *Handbook of Physiology*, eds. Knobil, E. & Sawyer, W. H. (American Physiological Society, Washington, DC), Vol. 4, pp. 45–80.
31. Manning, M., Gzronka, Z. & Sawyer, W. H. (1981) in *The Pituitary*, eds. Beardwell, C. & Robinson, G. (Butterworths, London), pp. 265–296.
32. Wynn, P. C., Aguilera, G., Morell, J. & Catt, K. J. (1983) *Biochem. Biophys. Res. Commun.* **110**, 602–608.
33. Gillies, G., Van Wimersma Greidanus, T. B. & Lowry, P. J. (1978) *Endocrinology* **103**, 528–534.
34. Woodcock, E. A. & Johnston, E. C. J. (1982) *Endocrinology* **111**, 1687–1691.
35. Jard, S., Cantau, B. & Jakobs, K. H. (1981) *J. Biol. Chem.* **256**, 2603–2606.
36. Capponi, A. M., Favrod-Coune, C. A., Gaillard, R. C. & Muller, A. F. (1982) *Endocrinology* **110**, 1043–1045.
37. Rodbell, M. (1980) *Nature (London)* **284**, 17–22.
38. Giguere, V. & Labrie, F. (1982) *Endocrinology* **111**, 1752–1754.
39. Westendorf, J. M., Philips, M. A. & Schonbrunn, A. (1983) *Endocrinology* **112**, 550–557.
40. Gaillard, R. C., Grossman, A., Gillies, G., Rees, L. & Besser, G. M. (1981) *Clin. Endocrinol. (Oxford)* **15**, 573–578.
41. Mormède, P. (1983) *Nature (London)* **302**, 345–346.