^{125}I-8-L-Arginine Vasopressin Binding to Human Mononuclear Phagocytes

LUTZ H. BLOCK, RUDOLF LOCHER, WERNER TENSCHERT, WALTER SIEGENTHALER, TRAUDE HOFMANN, RUTH METTLER, and WILHELM VETTER,
Department of Medicine, University of Zürich, CH-8091 Zürich, Switzerland

ABSTRACT The binding of vasopressin to human circulating blood cells was examined. Direct binding studies with preparations of single cell types indicated that the mononuclear phagocyte system is almost entirely responsible for binding of the hormone. Binding of ^{125}I-8-L-arginine vasopressin (AVP) (40 pM) in the presence of excess unlabeled hormone was saturable (2.8±0.4 fmol/2 × 10^6 cells per ml), was linear with cell number, was dependent upon the concentration of the radioligand used, and was reversible. Binding equilibrium was achieved in 30–40 min at 22°C. Scatchard analysis of binding at this time showed an apparent dissociation constant of 25±0.21 pM, providing an estimate of 640±80 sites/cell.

Pretreatment of the cells with cytochalasin B, an agent that can block phagocytosis, did not modify radioligand binding, which indicates that ^{125}I-AVP uptake by the cells is due to binding and not to endocytosis. Specificity of vasopressin-sensitive sites on mononuclear phagocytes was demonstrated with a series of vasopressin analogues with various degrees of antidiuretic potency, and with peptide hormones that bind to specific receptors on circulating blood cells but that lack antidiuretic activity.

AVP (40 pM) elevated the intracellular level of cyclic AMP from 137±8.6 to 350±20.5 pmol/mg cell protein. The binding affinities of the various analogues were correlated with their ability to stimulate intracellular cyclic AMP synthesis (Lys^8-vasopressin < deamino(8-D-Arg)-vasopressin < oxytocin).

INTRODUCTION

Specific hormone receptor sites on the membranes of various tissues have been characterized for a variety of hormones by means of direct binding experiments with radiolabeled ligands. Existence of specific binding sites for several hormones has been demonstrated not only in specific target tissues, but also in peripheral tissue, where actions of these hormones are unknown at a molecular level. Molecular alterations of human target tissue, which could be associated with the pathogenesis of certain diseases, can therefore be analyzed by comparing hormone action on target tissue in vivo with that of readily accessible peripheral tissue in vitro. For example, decreased insulin binding to adipocytes was shown to correlate with decreased binding to circulating monocytes from obese subjects (1). Thus, an estimation of direct insulin binding to monocytes in vitro could be indicative of insulin action in vivo (2).

It appears to be a valid generalization that the sensitivity of tissues varies inversely with the availability of hormone under different pathophysiological situations of the organism, and that often the altered sensitivity of tissues to exogenously administered hormone may be directly correlated to the receptor content of the tissues. For instance, insulin binding studies on human peripheral leukocytes have been used for the detection of insulin receptor defects in human insulin-resistant states (3, 4). Recently Williams et al. (5) reported the first successful application of direct binding studies to the identification of β-adrenergic receptors in human tissue by characterizing (−) [^{3}H]-alprenolol receptors in the human circulating lymphocyte. The clinical implications of such receptor studies using direct binding methods have been reviewed by Lefkowitz (6) and Baxter and Funder (7). There is evidence that receptors for both catecholamines and peptide hormones are localized on the membrane of various cell types (7). To achieve new physiologic and biochemical information on the action of vasopressin in various clinical situations, we were interested in characterizing specific receptors for the peptide in the human circulating blood cells by using direct binding studies.

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We report here the observation that the radioligand ^{125}I-8-L-arginine vasopressin (AVP)[1] is capable of binding to human mononuclear phagocytes, which may provide a useful tool for studies on abnormal receptor properties in human disease.

METHODS

Pharmacological agents. The radioligands ^{125}I-AVP, ^{125}I-Lys^8-vasopressin (LVP), ^{125}I-deamino (8-D-arginine) vasopressin (d-DAVP), and ^{125}I-oxytocin (sp act of 1,100–1,500 μCi/μg) were purchased from New England Nuclear (Boston, Mass.). Bovine serum albumin (fraction V), isoproterenol, 3-isobutyl-1-methylxanthine (IBMX), beef heart cyclic 3',5' phosphodiesterase, and cytochalasin B were obtained from Sigma Chemical Co. (St. Louis, Mo.). Bovine insulin was purchased from Serva Feinbiochemika GmbH & Co. (Heidelberg, Germany), angiotensin II, LVP, and oxytocin from Senn (Zürich, Switzerland), and ACTH from Ciba-Geigy Ltd. (Basel, Switzerland). AVP (purity > 98%), and d-DAVP were obtained from Ferring AB Pharmaceuticals (Malmö, Sweden). The chemically modified vasopressins (4-valine, 8-D-arginine) vasopressin, deamino (4-threonine, 8-D-arginine) vasopressin, and [1(β-mercapto-β,β-cyclopentamethylene propionic acid), 4-valine, 8-D-arginine] vasopressin were kindly provided by Dr. M. Manning, Medical College of Ohio (Toledo, Ohio) and Dr. C. Roy, College de France (Paris, France); and deamino-dicarba-arginine vasopressin was obtained from Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.

Preparation of mononuclear leukocytes. Individual units of 500 ml fresh human heparinized blood obtained from healthy donors were sedimented and the buffy coats aspirated. Mononuclear cells were isolated from the buffy coat cells in Ficoll-Hypaque gradients (8). After two washes in Hanks' buffer (Microbiological Associates, Walkersville, Md.) at room temperature, the cells were suspended in Eagle's minimal essential medium, containing 24 mM Hepes (Gibco Laboratories, Grand Island Biological Co., Grand Island, N. Y.). The viability of the cells was determined by trypan blue exclusion and was found to be <98%. This fraction contained 15% monocytes, 82% lymphocytes, and 3% granulocytes, as determined by differential cell counting on cell preparations, stained with Türk's solution.

Isolation of mononuclear phagocytes. For separation of mononuclear cell population, cells were incubated in plastic petri dishes (35 mm Diam; Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.) at a concentration of 1×10^7 cells/ml per dish for 1 h at 37°C in a 5% CO_2 atmosphere. After this incubation period, the nonadhering cells (containing lymphocytes and polymorphonuclear leukocytes) were decanted and the dishes gently rinsed with warm Eagle's minimal essential medium. The adhering cells were then removed by extensive washing with cold Eagle's minimal essential medium and the cells from several dishes were pooled, washed (10 min, 800 *g*, 4°C) with the assay buffer, and quantitated by the nonspecific esterase method (9). The mononuclear phagocyte content of this cell preparation was 88±1.7%. All cell counts were done in triplicate with the aid of a Neubauer chamber. All assays were done within hours after preparation of the cells, during which time the cells were kept on ice. Lymphocytes, polymorphonuclear leukocytes, and erythrocytes were prepared according to the methods described by Williams et al. (5).

^{125}I-AVP binding assay. For the binding assay, mononuclear phagocytes were suspended in the assay buffer containing 100 mM Hepes, 120 mM $MgSO_4$, 15 mM Na acetate, 1 mM EDTA, and 1% bovine serum albumin, pH 8. Cells at a concentration of 2×10^6 were incubated in a total volume of 1 ml with ^{125}I-AVP and unlabeled hormone in 5-ml plastic tubes (Falcon Labware) for various time intervals at 22°C, as described in the legends to our figures and tables. For competition experiments with the structural antidiuretic-hormone (ADH) analogues, various concentrations of the compounds were added as described below. The reaction was terminated by washing of the cells (5', 900 *g*, 4°C). The cell pellet was resuspended in 5 vol of buffer and washed twice (5', 900 *g*, 4°C) and radioactivity in the cell pellets was counted in a Packard Auto Gamma Scintillation Spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.) with efficiency of 50%. All assays were run in triplicate.

In each experiment total binding of ^{125}I-AVP to the cells was determined by incubating the cell preparations with the radioligand in the absence of unlabeled peptide and by measuring the amount of radioactivity retained on the cells. "Specific" binding of the radioligand was analyzed in a separate incubation by adding a 2.5-fold excess of unlabeled hormone, and was 85–95% of total ^{125}I-AVP bound to the cells. Values obtained for total binding minus specific binding values refer to "nonspecific" binding.

For the binding studies on cells from patients, only the mononuclear cell fraction was prepared from 80–100 ml heparinized blood. These studies included three patients with the hereditary type of diabetes insipidus (one male and two females), five male patients with maturity onset diabetes mellitus, and seven male normal donors. The hereditary type of diabetes insipidus was traced through four consecutive generations. Urine volumes of the patients before therapy (8.3–13.3 liters/d) and osmolality (85–145 mosmol/kg) changed to volumes in the range of 1.5–1.6 liters/d and to an osmolality of 500–780 mosmol/kg, when the patients were treated twice a day with 0.1 mg d-DAVP.

Binding of either ^{125}I-AVP (40 pM) or ^{125}I-insulin (33 pM); specific activity 160–180 μCi/μg prepared as described by Gambhir et al. (10) was determined according to DeFronzo et al. (2). Briefly, 3×10^6 mononuclear cells (containing 2.5×10^5 mononuclear phagocytes) were incubated in a final volume of 1 ml assay buffer in the presence or absence of unlabeled hormone. The nonspecific binding of ^{125}I-insulin was defined by the amount of ^{125}I-insulin bound to the cell pellet in the presence of 10^4 ng/ml of unlabeled insulin, and was found to amount to 0.3–0.5% of the total radioactivity added to the cells obtained from normal or diabetic subjects or from patients with the hereditary type of diabetes insipidus. The nonspecific binding of ^{125}I-AVP was determined in the presence of 100,000-fold excess of unlabeled peptide and was 0.3–0.7% of the total radioactivity added for all groups tested. The experiments were performed in duplicate.

Cyclic AMP measurements. To determine the intracellular level of cyclic AMP, accumulation measurements were performed in the presence of the phosphodiesterase inhibitor IBMX at a final concentration of 0.1 mM. Experimental treatment and processing of the cells for the cyclic AMP assay were performed as described by Higgins and David (11), except that the cells (8×10^6/ml) were incubated in Eagle's minimal essential medium in the absence of serum for various time intervals, as described below. At the end of the assay period 1 ml of ice-cold 10% trichloroacetic acid (which contained 0.1 pmol or ~2,000 cpm of [^{3}H]cyclic AMP to measure

[1] *Abbreviations used in this paper*: ADH, antidiuretic hormone; AVP, 8-L-arginine-vasopressin; d-DAVP, deamino (8-D-arginine) vasopressin; IBMX, 3-isobutyl-1-methylxanthine; LVP, Lys^8-vasopressin.

recovery) was added to 1 ml of the cell suspension. After boiling for 5 min and addition of 2 ml ethanol:30% HCl (60:1), the samples were homogenized (Polytron Kinematica, Lucerne, Switzerland) at maximum velocity for 30 s. The homogenates were centrifuged at 5,000 *g* for 15 min at 4°C. The pellets were solubilized in 2% sodium dodecyl sulfate and the protein content was determined according to Lowry et al. (12) by using bovine serum albumin (Sigma Chemical Co.) as a standard. The supernates were lyophilized and redissolved in sodium borate buffer (50 mM), pH 8.9, containing 0.1% gelatine.

To determine the intracellular cyclic AMP level, 100-μl aliquots were assayed by using the cyclic AMP (^{125}I) radioimmunoassay kit (New England Nuclear). Each sample was assayed in triplicate. The results are expressed as picomoles of cyclic AMP per milligram of protein (or ~1.1×10^7 mononuclear phagocytes). Vasopressin-induced cyclic AMP accumulation was further demonstrated by product identification, as incubations of the supernates with beef heart cyclic-3′,5′-phosphodiesterase (Sigma Chemical Co.) for 3 h at 30°C at pH 7.5 according to the method of Butcher and Sutherland (13) decreased the measurable cyclic AMP by 95%.

To substantiate the relationship between binding of the ligand and cyclic AMP accumulation, the cells were exposed to various concentrations of either ^{125}I-AVP, ^{125}I-LVP, ^{125}I-d-DAVP, or ^{125}I-oxytocin for 30 min at 37°C. After termination of the reaction by addition of 10% trichloroacetic acid, the cells were processed as described above. The supernates were analyzed for cyclic AMP after separation of the radioligands on ion-exchange columns (1-x, 200–400 mesh Bio-Rad Laboratories, Richmond Calif.) as described by Murad et al. (14).

To determine cyclic AMP recovery, ^{3}H-cyclic AMP (in the same amount as described above) was added to the experimental samples, which were then subjected to the column procedure. The recovery was found to be ~75%. The eluted fractions containing cyclic AMP were pooled, lyophilized, resuspended in 5 ml assay buffer, and analyzed for cyclic AMP content by using the cyclic AMP radioimmunoassay kit. A dose of 40 pM ^{125}I-AVP elevated the intracellular cyclic AMP level to 285±27.6 pmol/mg protein from a basal level of 110±13.5 pmol/mg protein. ^{125}I-LVP (40 pM) increased intracellular cyclic AMP levels to 235±14.7 pmol/mg protein, ^{125}I-d-DAVP (40 pM) to 205±19.5 pmol/mg protein, and ^{125}I-oxytocin (40 pM) to 175±18.8 pmol/mg protein from a basal level of 108±13.5 pmol/mg protein. As a positive control for cyclic AMP production, DL-isoproterenol (0.1 mM in the presence of IBMX) elevated intracellular cyclic AMP levels three to fourfold over the basal level (maximum between 5–10 min and back to basal level by ~40 min).

RESULTS

Cell type involved in vasopressin binding. ^{125}I-AVP appears to bind almost exclusively to mononuclear phagocytes, and much less binding is found for lymphocytes, granulocytes, or erythrocytes. As is shown in Table I, mononuclear phagocytes (88% pure) bound 2.8 fmol ^{125}I-AVP/2×10^6 cells per ml, whereas the specific binding of the radioligand to preparations of purified leukocytes (98% lymphocytes, 1.5% mononuclear phagocytes, and 0.5% polymorphonuclear leukocytes) was 0.2 fmol/2×10^6 cells. Preparations of highly purified polymorphonuclear leukocytes and erythrocytes revealed specific binding of the radiolabeled hormone between 0.2 and 0.09 fmol/2×10^6 cells per ml.

Binding of ^{125}I-AVP to human mononuclear phagocytes. The amount of vasopressin that binds to human mononuclear phagocytes is dependent on the time of incubation (Fig. 1). At a concentration of 40 pM ^{125}I-AVP, the amount of specifically bound hormone increases with time and reaches an equilibrium value of 10±0.5%/2×10^6 cells total binding within 30–40 min. Nonspecific binding under these conditions was determined in the presence of 100 pM of unlabeled peptide to be 1.5±0.12%. Thus, mononuclear phagocytes bind specifically 8.5±0.8%/2×10^6 cells.

To exclude the possibility that the radioligand binding was due to internalization by the mononuclear phagocytes, the cells were treated with cytochalasin B (10 μg/ml) to inhibit phagocytosis (15). No significant difference was found in specific binding of ^{125}I-AVP (40 pM) between those cells treated with and those without cytochalasin B, which indicates that the observed binding was not caused by endocytosis of the radioligand.

TABLE I

Specific Binding of ^{125}I-AVP to Preparations of Single Cell Populations of Human Blood

Isolated cell population	Lymphocytes	Mononuclear phagocytes	Polymorphonuclear leukocytes	Erythrocytes	Specifically bound ^{125}I-AVP (fmol/2×10^6 cells per ml)
	%	%	%	%	%
Mononuclear phagocytes	4±2.5	88±6.5	8±5.7	—	2.8±0.2
Lymphocytes	98±1.2	1.5±1.3	0.5±0.4	—	0.19±0.08
Polymorphonuclear leukocytes	1±0.6	2±1.2	97±3	1±0.8	0.21±0.06
Erythrocytes	—	—	1±0.4	99±0.8	<0.09

Cell populations were isolated from blood of healthy donors as described in Methods. Cells at a density of 2×10^6/ml were incubated with 40 pM of ^{125}I-AVP in the presence of 100 pM unlabeled hormone for 2 h at 22°C. Cells were stained with Türk's solution, and differential counts were done in triplicate for each individual experiment. Mononuclear phagocytes were stained by the nonspecific esterase method (9). The data represent the mean of triplicate incubations of three independent experiments (±SD).

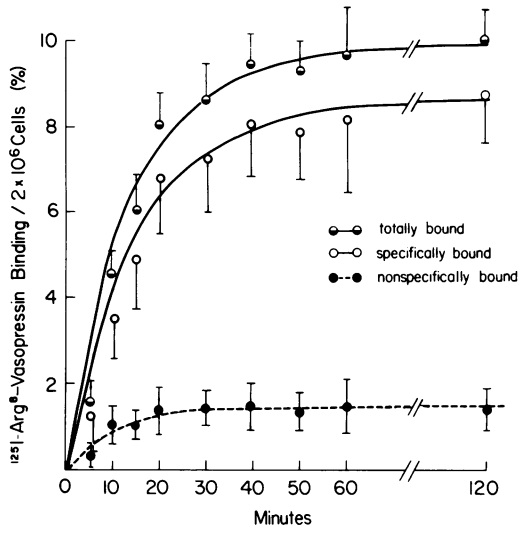

FIGURE 1 Specific binding of ^{125}I-AVP (Arg^8-vasopressin) as a function of time. Mononuclear phagocytes (2×10^6 cells/ml) were incubated with ^{125}I-AVP (40 pM) in the presence of excess unlabeled AVP (100 pM) at 22°C. Specific binding was determined at the indicated times, as described in Methods. The data represent the mean±SD of six independent experiments.

As is shown in Fig. 2, the binding of vasopressin varies as a function of the number of cells. With cell concentrations between 10^6 and 5×10^6 cells/ml, a linear increase in specific binding of ^{125}I-AVP (40 pM) was seen.

The binding of vasopressin was also dependent on the concentration of ^{125}I-AVP (Fig. 3). Significant

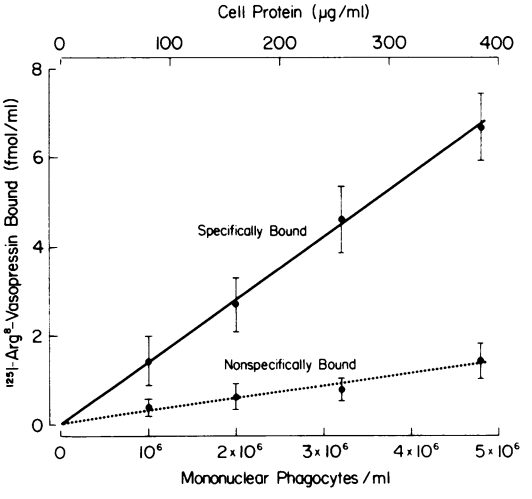

FIGURE 2 Relationship of cell concentration and cell protein to specific ^{125}I-AVP (Arg^8-vasopressin) binding. Various amounts of mononuclear phagocytes between 1 and 5×10^6 cells/ml were incubated with the radioligand (40 pM) for 120 min at 22°C. Specific binding was assessed in the presence of 100 pM unlabeled hormone. Each value shown represents the mean±SD of five independent experiments.

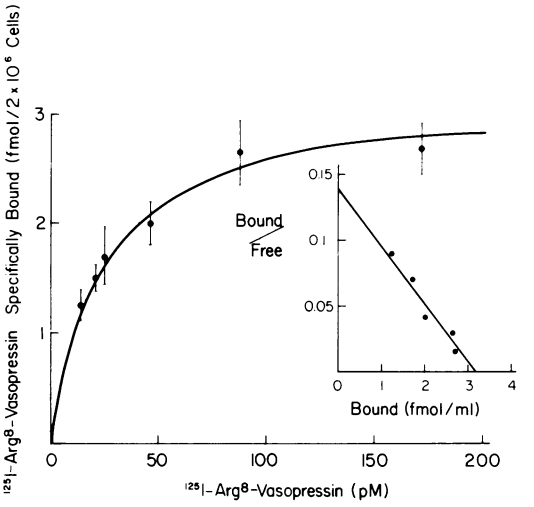

FIGURE 3 Specific binding as a function of concentration of ^{125}I-AVP (Arg^8-vasopressin). Mononuclear phagocytes (2×10^6 cells/ml) and various concentrations of the radioligand were incubated in the assay buffer for 2 h. Specific binding was determined at each concentration of ^{125}I-AVP. The data are the mean±SD of triplicate determinations derived from five independent experiments. The insert shows a Scatchard plot of ^{125}I-AVP binding to mononuclear phagocytes at a cell concentration of 3×10^6 cells/ml. The regression line ($r = 0.93$) indicates a K_d of 25±0.21 pM and a number of binding sites equivalent to 640±80 receptors cell.

binding was observed at 10 pM and complete saturation occurred at a concentration of 170 pM. Scatchard analysis (16) indicates an apparent dissociation constant (K_d) of binding of 25±0.21 pM (mean±SD, $n = 5$) and a number of 640±80 binding sites/cell.

To characterize the specificity of the vasopressin receptors, the cells were incubated with ^{125}I-AVP in the presence of varying amounts of unlabeled structural analogues, and the displacement of the radioligand was assessed. As is illustrated in Fig. 4, after exposure of the cells to ^{125}I-AVP (40 pM), the ligand was displaced by addition of increasing concentrations of unlabeled hormone or of analogues known to have almost identical binding kinetics in other systems, and to have a comparable antidiuretic potency or, as in the case of [1-(β-mercapto-β,β-cyclopentamethylene propionic acid), 4-valine, 8-D-arginine] vasopressin, to behave as a vasopressin antagonist (17). A comparison of the concentrations, which lead to ~50% inhibition of binding (ID_{50}), shows that the inhibitory concentration for LVP and deamino-dicarba-arginine vasopressin is ~10-fold, that for d-DAVP is 100-fold, and that for oxytocin is 1,000-fold higher than that for AVP.

To demonstrate that the competition of the analogues for ^{125}I-AVP binding to the ADH receptor sites was indeed specific, the dissociation constants for some of the compounds were determined and compared with that of ^{125}I-AVP. As is shown in Table II, there was good

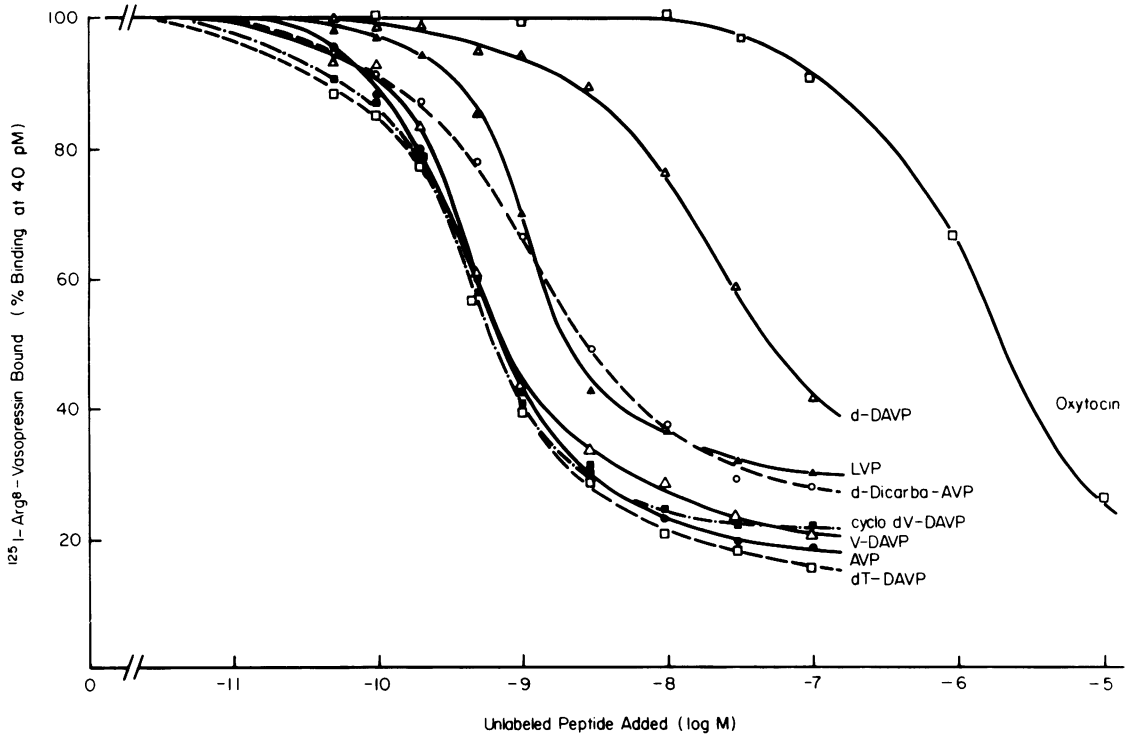

FIGURE 4 Specificity of mononuclear phagocyte receptors toward various ADH analogues. Binding of ^{125}I-AVP (Arg^8-vasopressin) (40 pM) after 1 h preincubation of the cells at 22°C was 2.9 fmol/2 × 10^6 cells per ml (100%). At zero time increasing amounts of the various ADH analogues were added for one additional hour, after which specific binding of the radioligand was assessed. The experimental values are the mean of three independent experiments.

agreement between the concentration reported for inhibition of ^{125}I-AVP binding of the structural analogues and their individual binding affinities to the ADH receptor. To analyze the specificity of ADH receptor sites further, the cells were incubated with the radioligand in the presence of increasing amounts of polypeptide hormones without antidiuretic activity. For example, insulin has been shown previously to bind to specific sites on human monocytes (2, 18). Although the addition of unlabeled AVP decreases radioligand binding as expected, angiotensin II, ACTH, and insulin do not modify the radioligand binding at all.

TABLE II
Binding Characteristics of Vasopressin Analogues to Human Mononuclear Phagocytes

Radioligand	Bound radioligand	K_d
	fmol/mg cell protein	*pM ± SD*
^{125}I-AVP	15.6±1.3	25±0.21
^{125}I-LVP	6.2±0.82	75±8.7
^{125}I-d-DAVP	4.6±0.45	89±7.1
^{125}I-Oxytocin	4.1±0.38	516±43.6

Mononuclear phagocytes (2 × 10^6/ml) were incubated in the presence of various concentrations of each radioligand in the assay buffer for 2 h at 22°C. Specific binding was determined at each concentration of either ^{125}I-AVP, or ^{125}I-LVP, or ^{125}I-d-DAVP, or ^{125}I-oxytocin. The K_d values are derived from Scatchard analysis of binding and are the mean±SD of triplicate determinations derived from four independent experiments.

Effect of vasopressin on the intracellular level of cyclic AMP in mononuclear phagocytes. Vasopressin has been demonstrated to bind to various cell types of different species with subsequent increase of the intracellular level of cyclic AMP (19–23). To investigate whether the neurohypophyseal peptide is capable of stimulating the production of cyclic AMP in human mononuclear phagocytes, cells were incubated with varying amounts of the hormone. Hydrolysis of endogenous cyclic AMP by phosphodiesterase was prevented by addition of the phosphodiesterase inhibitor IBMX (0.1 mM). Incubation of the cells with AVP (500 pM) for 30 min led to an increase of the intracellular level of cyclic AMP to 350±20.5 pmol/mg of cell protein (or 1.1 × 10^7 cells), as compared with the control of 137±8.6 pmol/mg of cell protein (or 1.1 × 10^7 cells) (Fig. 5).

If ADH binding to human mononuclear cells and elevation of the intracellular level of cyclic AMP are

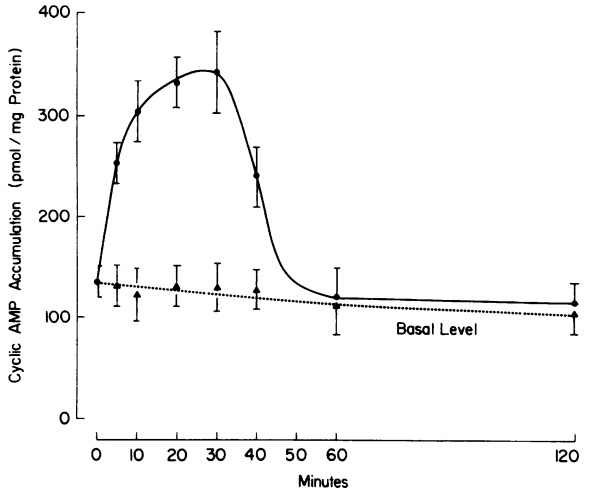

FIGURE 5 AVP-induced accumulation of cyclic AMP as a function of time. Mononuclear phagocytes (8×10^6/ml) were incubated with 40 pM unlabeled hormone for the indicated time intervals. Intracellular cyclic AMP levels are expressed in milligrams of cell protein per milliliter (equal to 1.1×10^7 cells). The values are the mean±SD derived from three independent experiments.

related, the structural analogues should increase the level of cyclic AMP at concentrations similar to those at which they competed for ^{125}I-AVP binding. As is illustrated in Fig. 6, AVP was by far the most active hormone in stimulating intracellular cyclic AMP synthesis, followed by LVP, d-DAVP, and oxytocin. The time-course for the elevation of intracellular cyclic AMP level by AVP (500 pM) revealed a significant increase after 5 min of incubation, peaking at 15–30 min and returning to basal values at 40–120 min (Fig. 5). The structural analogues showed a similar time-course (data not shown).

Clinical aspects of ^{125}I-AVP binding studies. To investigate direct binding of ^{125}I-AVP to peripheral blood cells in certain clinical situations, we studied its binding in patients with the lower levels of vasopressin that have been observed in patients with the hereditary type of diabetes insipidus. The binding of ^{125}I-AVP to cells of patients with the hereditary type of diabetes insipidus ($n = 3$) was increased to 6±0.8 fmol/5×10^6 cells per ml, when compared with its binding to cells of patients with maturity onset diabetes mellitus (4.15±0.4/5×10^6 cells per ml, $n = 5$) and to cells of normal donors (4.3±0.5/5×10^6 per ml, $n = 7$). In contrast, the binding of ^{125}I-insulin to cells of patients with hereditary type of diabetes insipidus (8.75±1.2 pg/5×10^6 cells per ml) and to cells of normal donors (9.2±0.7/5×10^6 per ml) appears to be almost the same, whereas a decrease in insulin binding was observed in cells of diabetics (6.2±0.8/5×10^6 cells per ml).

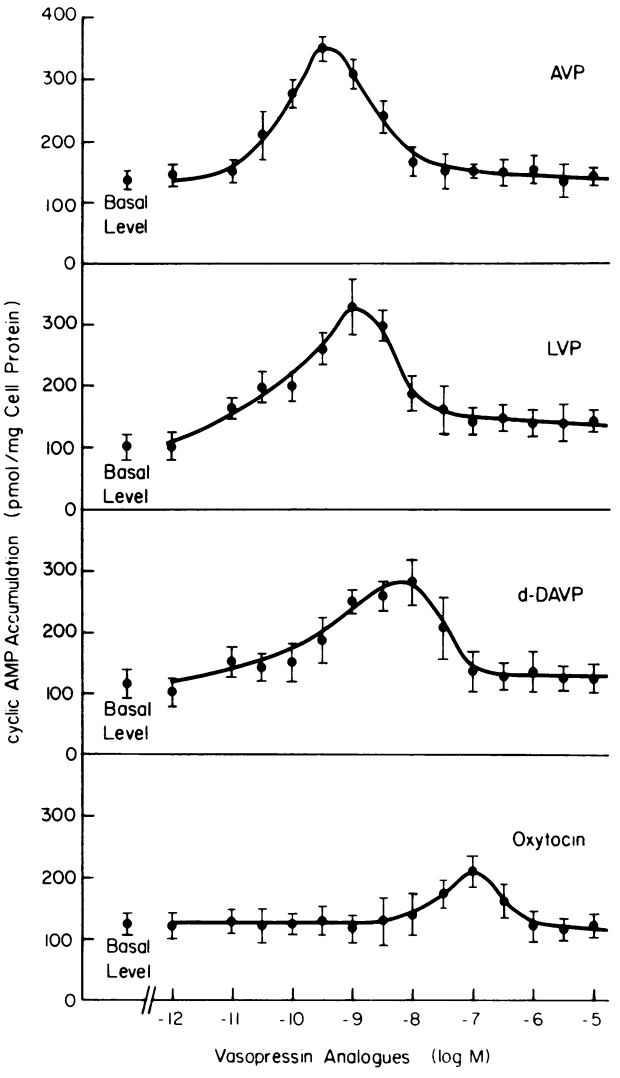

FIGURE 6 Stimulation of cyclic AMP accumulation by various concentrations of AVP, LVP, d-DAVP, and oxytocin in mononuclear phagocytes. Cells (8×10^6/ml) were incubated for 30 min at 37°C with various concentrations of the unlabeled hormone. Cyclic AMP content in the cells was determined in the presence of IBMX (0.1 mM), as described in Methods, and is expressed in milligrams of cell protein per milliliter (equal to 1.1×10^7 cells). Intracellular cyclic AMP accumulation without IBMX was 207±14.5 pmol/mg protein per ml. Data are the mean±SD of six independent experiments.

DISCUSSION

Our binding studies with vasopressin using circulating cells of human blood show that the mononuclear phagocyte system appears almost entirely responsible for the interaction with the hormone. The highly purified mononuclear phagocytes (88% purity) bound specifically 94% of the radioactively labeled hormone,

whereas highly purified lymphocytes (98% purity) and polymorphonuclear leukocytes (97% purity) bound < 6% each, and erythrocytes (99% purity) < 3%. Thus, the specific binding of vasopressin can be almost entirely accounted for by binding to mononuclear phagocytes. The observation that treatment of the cells with the phagocytosis blocking-agent cytochalasin B does not affect the radioligand binding significantly suggests that the observed binding was not due to internalization of the hormone.

Studying the binding parameters of vasopressin to human mononuclear phagocytes, we find that specific binding is saturable with time, is linear with cell number, is dependent on the concentrations of the ligand used, and is reversible. The analysis of binding at equilibrium revealed an apparent K_d of 25 pM, corresponding to 640±80 sites/cell. A comparable number of ~2,000 sites/cell has recently been determined in human lymphocytes for the β-adrenergic antagonist (−) [^{3}H]alprenolol (5). A similar number of 1,800 sites has been reported for β-adrenergic antagonists in amphibian erythrocytes (24). According to Butlen et al. (17), the K_d value for vasopressin binding to medullopapillary portions of the rat kidney is 390 pM. Thus, the K_d value for vasopressin binding to human mononuclear phagocytes is in a comparable range with the K_d value obtained from membrane preparations of the rat kidney.

Previous studies have shown that the ADH receptors from various species discriminate among neurohypophyseal peptides and analogues of closely related structures (19–23). When comparing the individually obtained K_d values of LVP, d-DAVP, and oxytocin with their ability to compete for ^{125}I-AVP binding, we find good agreement between these two sets of data. Furthermore, the vasopressin antagonist [1(β-mercapto-β,β-cyclopentamethylene propionic acid), 4-valine, 8-D-arginine] vasopressin has a K_d value similar to AVP, and has been shown to completely inhibit the antidiuretic action of LVP in the rat system (17). It is almost as effective in decreasing ^{125}I-AVP binding as AVP. On the other hand, insulin, which has been reported to act on specific sites on human monocytes and erythrocytes (1, 2, 10), angiotensin II, and ACTH —all of which lack antidiuretic activity—did not interfere with radioligand binding. These observations strongly suggest specificity for hormonal binding.

Various studies have shown the existence of specific sites for certain ligands on the mononuclear phagocytes capable of affecting cyclic AMP metabolism in these cells (25). By comparing the concentrations of the hormone that lead to maximal stimulation of endogenous cyclic AMP accumulation in the mononuclear phagocytes with those required for maximal specific binding, we find that the effective concentrations in both experiments are in a similar range: between 100 and 1,000 pM. The binding affinities of LVP, d-DAVP, and oxytocin paralleled the required concentrations of these agents to stimulate intracellular cyclic AMP production in mononuclear phagocytes. The observation that optimum concentrations of vasopressin and its analogues are required to give a maximum response in intracellular levels of cyclic AMP, whereas higher concentrations of AVP were less effective, is in agreement with a report by Schwartz et al. (26), who described a biphasic phenomenon for vasopressin-induced intracellular accumulation of cyclic AMP in epithelial cells from the collecting bovine duct of the kidney. Though various explanations for the biphasic effect of vasopressin on cyclic AMP metabolism in mononuclear phagocytes are possible (e.g., changes in coupling between receptors and adenylate cyclase, with subsequent changes in cyclic AMP synthesis in the presence of high concentrations of the hormone; or alterations in ion fluxes caused by unphysiologic concentrations of the ADH), at present we do not have a mechanical explanation for this phenomenon. Another discrepancy was seen when the effective concentration of vasopressin leading to maximum saturation of its receptors (120–170 pM) was compared with that causing maximum stimulation of cyclic AMP synthesis (500 pM). This discrepancy may indicate that only a small fraction of specific vasopressin receptors is necessary to produce maximum stimulation of cyclic AMP synthesis in mononuclear phagocytes. Thus, it is possible that the already described "receptor-reserve phenomenon" for vasopressin is operative not only in the kidney but also in the mononuclear phagocytes of the circulating blood. A significant increase was seen in binding of the hormone to peripheral blood cells from patients with the hereditary type of diabetes insipidus when compared with patients with diabetes mellitus or with normal individuals. This increase in binding may result in the increased tissue sensitivity to AVP observed in patients with the hypothalamic dysfunction syndrome (27). This increase in binding is not due to changes in membrane properties of the cells in these patients, as the binding of insulin was unchanged in comparison with peripheral cells from normal individuals. The data indicate that the ADH receptor on human mononuclear phagocytes is distinct from other hormone receptors. It will be interesting to apply the method described here to peripheral blood cells of various types of diabetes insipidus and to investigate its use as a potential diagnostic tool.

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