Interactions between 8-L-Arginine Vasopressin and Prostaglandin E_2 in Human Mononuclear Phagocytes

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ABSTRACT The effect of 8-L-arginine vasopressin (AVP) on biosynthesis of prostaglandins in human mononuclear phagocytes was examined. AVP, oxytocin, and deamino-(8-D-arginine) vasopressin (dDAVP) affected prostaglandin biosynthesis in a rank order that parallels their pressor but not antidiuretic activity (AVP > oxytocin > dDAVP). Radioimmunoassay, incorporation studies using [^{14}C]arachidonic acid and radiometric thin-layer chromatography, revealed prostaglandin E_2 (PGE_2) to be the only prostaglandin synthesized by the mononuclear phagocytes.

While high concentrations of PGE_2 elevated cytoplasmic levels of cyclic AMP by five- to sevenfold above basal values, low concentrations of PGE_2 that are released by the cells in the presence of AVP failed to increase cyclic AMP content in the cells. However, PGE_2 at concentrations that do not alter cyclic AMP levels markedly interferes with the activity of AVP. This effect is, however, very time dependent. Addition of PGE_2 to the cells 30 min before AVP, was followed by a period of unresponsiveness to the hormone that lasts at least 30 min. Pretreatment of the cells with indomethacin enhanced the AVP-mediated accumulation of intracellular cyclic AMP level. PGE_2 did not modify [^{3}H]AVP binding, indicating that its inhibitory effect on the activity of the peptide is not due to down-regulation of vasopressin receptors.

INTRODUCTION

We have recently characterized specific 8-L-arginine vasopressin (AVP)[1] receptors on the circulating mononuclear phagocytes of human blood by using direct binding studies with [^{125}I]-AVP (1). An important observation in these studies was that AVP affects cyclic AMP metabolism of these cells at comparable concentrations that are effective in mediating the activity of AVP on osmotic water flow in toad bladder as well as in mammalian collecting duct.

It is well established that the increase in permeability to water that AVP elicits in both the renal collecting duct and the toad urinary bladder involves stimulation of the formation of cyclic AMP (2). Low concentrations of prostaglandin (PG) E_2 have been shown to antagonize the antidiuretic activity of AVP by inhibiting the accumulation of cyclic AMP in epithelial cells of the toad bladder (3) and in slices of renal medulla (4). In addition to its stimulatory effect on intracellular cyclic AMP level, AVP was also shown to increase biosynthesis of PGE_2 in cultured interstitial cells from renal medulla of rabbit (5). Thus, a short-loop feedback system has been proposed in which AVP itself stimulates its antagonist PGE_2 in collecting duct epithelial cells (6–8).

To evaluate whether human mononuclear phagocytes display certain characteristics of AVP action described for the renal tissue, we determined the effect of the hormone on PGE_2 biosynthesis. Further, we studied the interactions between AVP and PGE_2 on cyclic AMP metabolism of these cells.

METHODS

Pharmacological agents. AVP and deamino-(8-D-arginine) vasopressin (dDAVP) were purchased from Ferring AB Pharmaceuticals (Malmö, Sweden), Lys^8-vasopressin and oxytocin from Senn (Zürich, Switzerland). (1[β-mercapto-β,β-cyclopentamethylene propionic acid], 4-valine, 8-D-arginine) vasopressin (cyclo VAVP) was kindly provided by Dr. M. Manning, Medical College of Ohio (Toledo, OH). *dl*-Isoproterenol, 3-isobutyl-1-methylxanthine (IBMX), beef heart cyclic 3′,5′-phosphodiesterase, and indomethacin were obtained from Sigma Chemical Co. (St. Louis, MO). (8-L-arginine), [3-phenyl-3,4,5-^{3}H(N)]vasopressin ([^{3}H]AVF; 45.3 Ci/mmol sp act), [^{3}H]arachidonic acid ([^{3}H]AA); 78.2 Ci/mmol sp act) and [^{14}C]AA (56.3 Ci/mmol sp act) were pur-

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[1] *Abbreviations used in this paper:* AA, arachidonic acid; AVP, 8-L-arginine vasopressin; cyclo VAVP, (1[β-mercapto-β,β-cyclopentamethylene propionic acid], 4-valine, 8-D-arginine); dDAVP, deamino-(8-D-arginine) vasopressin; IBMX, 3-isobutyl-1-methylxanthine; PG, prostaglandin; TLC, thin layer chromatography; Tx, thromboxane.

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chased from New England Nuclear (Boston, MA). PGE_2, $PGF_{2\alpha}$, and thromboxane (TxB_2) were obtained from Upjohn Co., Kalamazoo, MI).

Preparation of mononuclear phagocytes. Individual units of 500 ml of 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 (9). 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, NY). The viability of the cells was determined by trypan blue exclusion and was found to be >97%. This fraction contained 16% monocytes, 80% lymphocytes, and 4% granulocytes, as determined by differential cell counting on cell preparation stained with Türk's solution.

For separation of mononuclear phagocytes, cells were incubated in plastic petri dishes (35-mm diam; Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, CA) 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, 400 *g*, 4°C) with the assay buffer, and quantitated by the nonspecific esterase method (10). The mononuclear phagocyte content of this cell preparation was 86±7.7%. 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.

Cyclic AMP accumulation 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 previously described (1). PGE_2 was dissolved in 0.2 M phosphate buffer and stored at −70°C. 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). The pellets were solubilized in 2% sodium dodecyl sulfate and the protein content was determined according to Lowry et al. (11) by using bovine serum albumin (Sigma Chemical Co.) as a standard. 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 (12), decreased the measurable cyclic AMP by 98%.

Determination of PGE_2 biosynthesis. To assess PGE_2 biosynthesis of mononuclear phagocytes in presence of AVP and analogues, the cells were suspended in RPMI 1640 medium (Flow Laboratories, Irvine, Ayrshire, England) containing 50 U/ml of penicillin and 50 μg of streptomycin at a concentration of 2×10^6 cells/ml per dish. The peptides were stored as stock solutions of 1 mM saline adjusted to pH 3.5 with acetic acid. Equivalent volumes of the peptides, isotonic saline plus acetic acid, and indomethacin (1 μg/ml) serving as controls were added to the cultures. Cells were incubated for various time intervals at 37°C in a 5% CO_2 atmosphere. The incubations were terminated by collecting the culture fluids and centrifuging them at 500 *g* for 10 min at 4°C. The resulting supernates were kept frozen at −70°C until measurements of prostaglandin biosynthesis were performed. Before use, all media were routinely assayed for presence of prostaglandins.

PGE_2 biosynthesis was estimated by using PGE radioimmunoassay kit (Clinical Assays Inc., Div. of Travenol Laboratories, Cambridge, MA) after isolation and separation of PGE_2 from the culture fluids by silicic acid chromatography in columns. For calculation of recovery, 10,000 cpm (~20 pg of [^{3}H]PGE_2) was added to 1 ml of culture fluids. [^{3}H]PGE_2 was recovered by 66±12.1%. 1-ml aliquots of cell supernates were extracted with 3 ml of ethylacetate, isopropanol, and 0.1 M HCl (3:3:1, vol/vol/vol) in polypropylene tubes (10 ml). After vortexing twice, the phases were separated by centrifugation (1 min, 400 *g*, 4°C). The organic phase (2.5 of 3 ml) was then dried at 37°C in nitrogen. Silicic acid chromatography in columns was performed according to Jaffé and Behrman (13). The eluates were dried in nitrogen and the residues redissolved in 1 ml of PGE_2-radioimmunoassay buffer. After adjustment at pH 12.5–12.8 by 0.1 ml of 1 M NaOH, the solution was boiled in tightly closed screw-capped vials, leading to conversion of PGE into PGB. After readjustment of pH to 7.4 with 10% (vol/vol) acetic acid, PGB was determined by radioimmunoassay. Biosynthesis of $PGF_{2\alpha}$ and TxB_2 was assayed by radioimmunoassays (Clinical Assays, Inc. and New England Nuclear). Radioactivity was measured in a liquid scintillation spectrometer.

To analyze prostaglandin end products in the cell supernates, mononuclear phagocytes (1.35×10^6/ml per dish) were incubated with [^{14}C]AA (1 μCi, New England Nuclear) in the presence or absence of indomethacin (1 μg/ml) for 2 h at 37°C in a 5% CO_2 atmosphere. The culture fluids were then collected, cleared by centrifugation (10 min, 400 *g*, 4°C), acidified to pH 3.0 with 0.5 N HCl, and extracted twice with 3 vol of ethylacetate. After evaporation of ethylacetate, the residues were dissolved in ethanol, and extracts (50 μl vol) were applied onto silicic acid thin-layer chromatography (TLC) plates (TLC aluminium sheets silica gel 60 F 254, Merck AG, Darmstadt, West Germany). $PGF_{2\alpha}$, PGE_2, TxB_2, and AA were used as standards. After being developed twice in the organic phase of ethylacetate/isooctane/acetic acid/water (11:5:2:1, vol/vol), the prostaglandin end products were visualized by exposing the TLC plates to iodine vapor. The silica gel was divided into 30 segments, scraped off, transferred to scintillation vials, and counted in a liquid scintillation counter.

To determine the effect of AVP on biosynthesis of radioactive PGE by mononuclear phagocytes, the cells (2×10^6/ml per dish) were incubated in the presence of [^{3}H]AA (1 μCi) for 14 h at 37°C in a 5% CO_2 atmosphere. After this incubation period, ~40% of radioactivity had been incorporated into lipids of the cells. The cells were then washed twice with warm RPMI 1640 and redissolved in 1 ml of medium. For experimental treatment, the cells were incubated in presence of indomethacin (1 μg/ml) plus AVP for two additional hours at 37°C in 5% CO_2. After collection of culture fluids, acidification, and extraction, TLC was performed as described above. After the cell supernates were decanted, 0.2 μCi each of [^{14}C]AA and of [^{14}C]PGE_2 were added to the cells to calculate recovery.

Experimental values are corrected for recovery of the ^{14}C-labeled standards. All experiments were performed in triplicate.

[^{3}H]AVP binding assay. To evaluate whether PGE affect [^{3}H]AVP binding to mononuclear phagocytes, the cells were

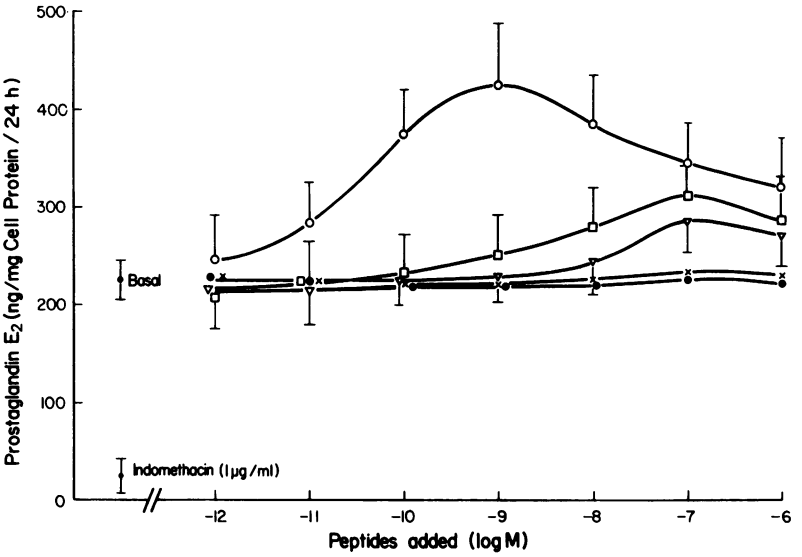

FIGURE 1 Effect of AVP (O —— O) and its analogues on PGE_2 biosynthesis in human mononuclear phagocytes. Cells (1.1×10^7/ml) were incubated in the presence of various concentrations of the hormones at 37°C in a 5% CO_2 atmosphere for 24 h. The effect of cyclo VAVP (● —— ●) was determined in absence and presence of AVP after a 30 min preincubation period of the cells. The incubation was stopped by collecting the cell supernates and clearing them by centrifugation. PGE_2 was determined by radioimmunoassay as described in Methods. The data represent the mean of triplicate incubations of five independent experiments (±SD). □ —— □, oxytocin; ▽ —— ▽, dDAVP; × —— ×, cyclo VAVP + AVP (500 pM).

suspended in the assay buffer containing 100 mM Hepes, 120 mM NaCl, 15 mM sodium acetate, 1 mM EDTA, and 1% bovine serum albumin, pH 8. [^{3}H]AVP binding assay was performed as previously described (1). "Specific" binding of the radioligand was analyzed in the presence of a 500-fold excess of unlabeled hormone, and was 80–85% of total [^{3}H]AVP bound to the cells. Values obtained for total binding minus specific binding values refer to "nonspecific" binding. The experiments were performed in triplicate.

RESULTS

The effect of AVP on PGE_2 levels in human mononuclear phagocytes. To demonstrate the ability of AVP to increase PGE_2 biosynthesis in human mononuclear phagocytes, the cells were incubated in the presence of varying concentrations of the peptide and its analogs. While AVP (500 pM) stimulated PGE_2 biosynthesis from 220±37.7 to 425±67.5 ng/mg protein per 24 h, effective concentrations of oxytocin (10^{-7} M) increased the production of PGE_2 to 315±42.8 ng/mg protein per 24 h and of dDAVP (10^{-7} M) to 285±52 ng/mg protein per 24 h (Fig. 1). Thus, the efficacy of AVP and its analogs to stimulate biosynthesis of PGE_2 corresponds to the rank order of pressor activity of the polypeptides (AVP > oxytocin > dDAVP; (14, 15). Preincubation of the cells with cyclo VAVP (5 μM), a specific antagonist of vasopressin's pressor activity, a compound that by itself is ineffective in stimulating PGE_2 at various concentrations tested (15), led to inhibition of PGE_2 biosynthesis in the mononuclear phagocytes when subsequently exposed to AVP (500 pM). As shown in Fig. 2, AVP increases PGE_2 levels

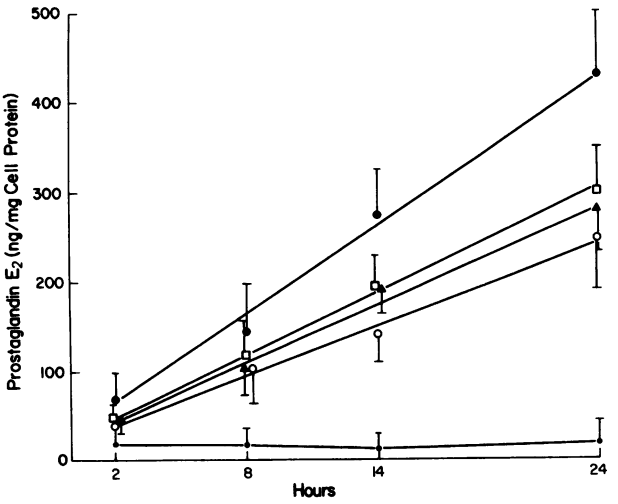

FIGURE 2 PGE_2 biosynthesis as a function of time. The cells (1.1×10^7/ml) were incubated in the presence and absence of AVP (● —— ●, 500 pM) and its analogs and in the presence of indomethacin (● —— ●, 1 μg/ml) for the indicated times. Experimental values represent the mean of triplicate incubations of four independent experiments (±SD, $P < 0.01$). □ —— □, 10^{-7} M oxytocin; ▲ —— ▲, 10^{-7} M dDAVP; o —— o, basal.

in the mononuclear phagocytes as a function of time. In addition, AVP stimulated PGE_2 synthesis by 50% over a cell protein range between 95 μg and 1 mg.

Comparison of the experimental values obtained for biosynthesis of $PGF_{2\alpha}$ and TxB_2 with that for PGE_2 in the cell supernatants, by radioimmunoassay after incubation with AVP, indicates that PGE_2 was virtually the only prostaglandin released by the mononuclear phagocytes (Table I). This was further substantiated by radiometric TLC experiments in which the mononuclear phagocytes were incubated with [^{14}C]AA. As shown in Fig. 3, there was only one radioactive peak that comigrated with authentic PGE_2. Similarly, when using cells which had [^{3}H]AA incorporated into cellular lipids, AVP increased the release of radioactive PGE_2 and AA but not that of any other product of fatty acid cyclooxygenase (Fig. 4).

The effect of PGE_2 on cyclic AMP metabolism of human mononuclear phagocytes. Prostaglandins have been shown to be formed within AVP-sensitive tissues (16, 17). To study whether PGE_2 affects cyclic AMP metabolism of mononuclear phagocytes, varying amounts of PGE_2 were added to the cells and accumulation of intracellular cyclic AMP was determined. As shown in Fig. 5, high concentrations of PGE_2 (10^{-4} M) increased intracellular cyclic AMP level by approximately five- to sevenfold above those that were achieved in the presence of AVP (500 pM) and approximately threefold above those that were caused by the β-adrenergic agonist *dl*-isoproterenol (10^{-4} M). However, when using low concentrations of PGE_2 that are released by cells after incubations with AVP (at concentrations that are effective in vivo), intracellular cyclic AMP level did not significantly increase above basal values.

There was no significant increase in PGE_2 biosynthesis and cyclic AMP accumulation in the presence

TABLE I
AVP-induced Biosynthesis of Prostaglandins by Human Mononuclear Phagocytes

Prostaglandins	Biosynthesis in the presence of AVP	Basal level	Biosynthesis in the presence of indomethacin (1 μg/ml)
		at 24 h/mg protein	
PGE_2	425±67.5 ng	250±37.7 ng	40±15 ng
$PGF_{2\alpha}$	195±25 pg	180±15 pg	—
TxB_2	110±16 pg	80±9 pg	—

Mononuclear phagocytes (2×10^6/ml) were incubated for 24 h in the presence and absence of AVP (500 pM) at 37°C in a 5% CO_2 atmosphere in plastic petri dishes. The incubation was determined by collection of the culture fluids and clearing them by centrifugation (10 min, 400 *g*, 4°C). Biosynthesis of prostaglandins was analyzed as described in Methods. The data are expressed per milligram cell protein (equal to 1.1×10^7 cells) and represent the mean of triplicate incubations of four independent experiments (±SD).

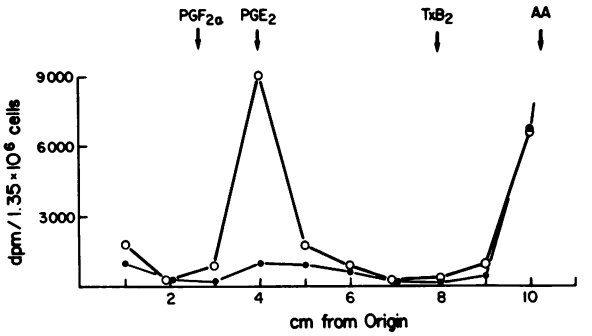

FIGURE 3 Thin layer chromatogram of [^{14}C]AA metabolites synthesized by human mononuclear phagocytes. Cells (1.35×10^6 ml per dish) were incubated for 2 h at 37°C in a 5% CO_2 atmosphere with [^{14}C]AA (1 μCi) and in the presence and absence of indomethacin. After collection of the culture fluids and extraction of the organic phase, the extracts were applied onto TLC plates and developed twice as described in Methods. PGF_2, PGE_2, TxB_2, and AA were used as standards. The data are the mean of three independent experiments. O —— O, control; ● —— ●, 1 μg/ml indomethacin.

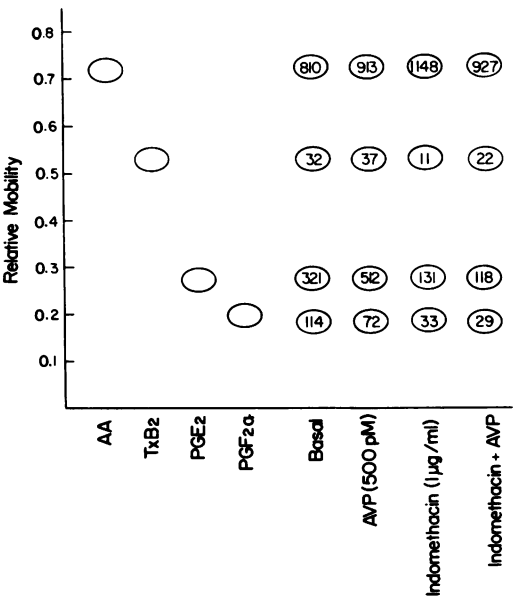

FIGURE 4 Facsimile of a thin-layer chromatogram of [^{3}H]AA metabolites that were incorporated into lipids of mononuclear phagocytes. Cells (2×10^6/ml per dish) were preincubated for 14 h at 37°C in a 5% CO_2 atmosphere in the presence of [^{3}H]AA before the addition of either AVP, or indomethacin, or AVP plus indomethacin. For experimental details see Methods. Open circles represent relative mobility of AA, TxB_2, PGE_2, and $PGF_{2\alpha}$. Experimental values are radioactivity in disintegration per minute. The data represent the mean of three independent experiments.

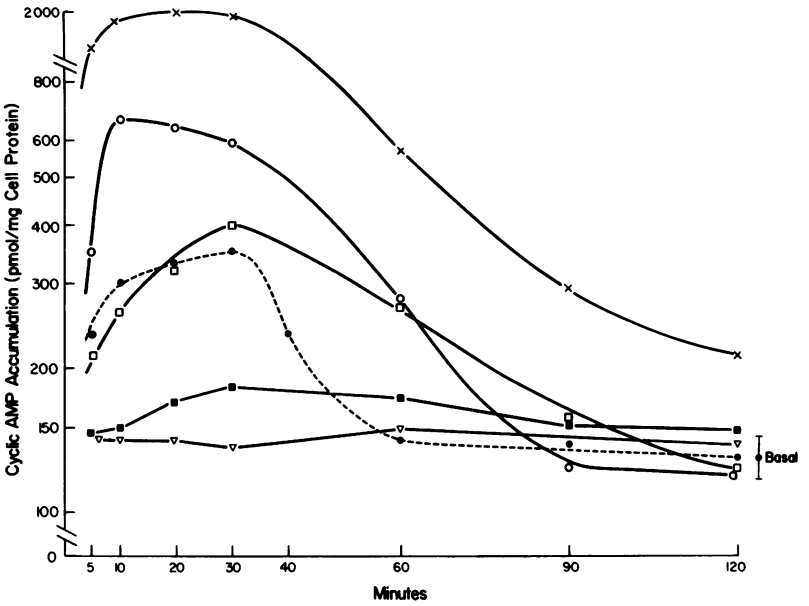

FIGURE 5 Stimulation of cyclic AMP accumulation by various concentrations of PGE_2, AVP, and *dl*-isoproteronol (10^{-4} M, O —— O) in human mononuclear phagocytes. Cells (8×10^6/ml) were incubated for 30 min at 37°C with the agents. Cyclic AMP content in the cells was determined in the presence of IBMX (0.1 mM), as described in Methods, and is expressed in milligrams of cell protein per milliliter. Data are the mean±SD of four independent experiments. ● - - - ●, 500 pM AVP; × —— ×, 10^{-4} M PGE_2; □ —— □, 10^{-7} M PGE_2; ■ —— ■, 10^{-8} M PGE_2; ▽ —— ▽, 10^{-9} M PGE_2.

of AVP in highly purified T and B lymphocytes (purity by 95%), thrombocytes (purity by 90%), polymorphonuclear leukocytes (purity by 96%), erythrocytes (purity by 97%), and basophils isolated from a patient with chronic myelogenous leukemia (purity by 65%) (data not shown).

Antagonism between PGE_2 and AVP on intracellular cyclic AMP level of human mononuclear phagocytes. Low concentrations of PGE_2 have been reported to inhibit AVP-induced cyclic AMP metabolism in various tissues (18). To evaluate whether PGE_2 antagonizes the AVP-dependent raise in intracellular cyclic AMP in human mononuclear phagocytes, the cells were preincubated for various time intervals with PGE_2 (10^{-9} M) before the addition of AVP (500 pM) for an additional 30 min. If 30 min were permitted to elapse before the addition of AVP, the inhibitory effect of PGE_2 on accumulation of intracellular cyclic AMP due to AVP was most pronounced (Table II). The greatly reduced response to AVP by a pulse of PGE_2 could almost be diminished by extensive washing of the cells (three times for 10 min). Little, if any, interference with the activity of AVP on cyclic AMP metabolism of mononuclear phagocytes was seen when the peptide was added either simultaneously or before the addition of PGE_2.

To study the effect of indomethacin on AVP-mediated cyclic AMP accumulation in mononuclear phagocytes, the cells were incubated for 30 min in the presence and absence of indomethacin before addition of AVP. As shown in Fig. 6, elevation of cytoplasmic level of cyclic AMP by AVP was increased to 405±51.7 fmol/mg protein in the presence of indomethacin peaking between 15 and 45 min and returning to basal level at 120 min as compared with cells incubated in the absence of indomethacin (302±37.4 fmol/mg protein) peaking at 15 min and returning to basal values at 60 min.

Lack of interference of PGE_2 on AVP binding to human mononuclear phagocytes. Since AVP binding and elevation of intracellular concentrations of cyclic AMP in mononuclear phagocytes are related, it appeared possible that PGE_2 interferes with AVP binding to these cells. We therefore determined specific [^{3}H]AVP binding to intact cells in the presence of various concentrations of PGE_2, $PGF_{2\alpha}$, and TxB_2. However, the PGE did not significantly modify the [^{3}H]AVP binding activity (4.2±0.7–4.6±0.5 pmol per 1.5×10^6 cells/ml in the presence of 100–1,000 ng PGE_2, 4.0±0.8–4.5±0.7 pmol per 1.5×10^6 cells/ml in the presence of 100 pg–1 μg $PGF_{2\alpha}$, and 4.4±0.4–4.7±0.5 pmol per 1.5×10^6 cells per ml in the presence of TxB_2) as compared with values determined in the absence of prostaglandins (4.2±0.6 pmol per 1.5×10^6 cells/

TABLE II

Interference with the Activity of AVP by PGE_2 on Accumulation of Intracellular Cyclic AMP Level in Human Mononuclear Phagocytes

Additions and procedures	Accumulation of intracellular cyclic AMP
	pmol/mg cell protein per 30 min
Basal level without IBMX	139±13.6
Basal level with IBMX (10^{-4} M)	154±14.8
PGE_2 (10^{-9} M)	163±10.3
AVP (10^{-9} M)	315±49.5
AVP + PGE_2	
0′	294±39.4
+15′	237±36.7
+30′	219±22.9
+ Pretreatment with PGE_2	
−15′	150±12.6
−30′	143±13.1

Time elapsed (−, minutes before and +, minutes after) between the addition of PGE_2 and the addition of AVP. AVP and PGE_2 were added to the cells (2×10^6/ml) at the indicated times in the presence of IBMX at 37°C. The incubations were continued for the usual 30-min period. Cyclic AMP content in the cells is expressed in milligrams of cell protein per milliliter (equal to 1.1 $\times 10^7$ cells). IBMX was present in all incubations. Extracellular cyclic AMP was estimated in the incubation medium at the same time points as when the cells manifested elevated intracellular levels and occasionally at subsequent time points when the intracellular accumulation was at or below control levels. Extracellular cyclic AMP level did not increase above 5–15% of basal values. The experimental values are the mean of triplicate incubations of four independent experiments (±SD).

ml). An additional experiment showing the displacement of [^{3}H]AVP binding by AVP analogues in presence of PGE_2, $PGF_{2\alpha}$, and TxB_2 did not result in changes of the displacement of binding by the analogues (data not shown).

DISCUSSION

AVP and its analogues have been reported to increase PGE_2 biosynthesis in various tissues (7, 19). The stimulation of renal biosynthesis of PGE_2 by AVP in mammalian kidney, however, is difficult to evaluate, for only urinary PGE have been determined. Urinary PGE include a portion of circulating PGE that were filtered or secreted into tubules as well as PGE that were synthesized within the kidney (20).

Although one cannot formally exclude the possibility that small numbers of cells could be responsible for the reported effects of our studies, we conclude that mononuclear phagocytes contribute to the release of PGE_2 into circulation. This is evidenced from the observation that highly purified T and B lymphocytes, polymorphonuclear leucocytes, erythrocytes, thrombocytes, and basophils lacked significant response to AVP. In addition, highly purified mononuclear phagocytes from human circulating blood and mouse peritoneal macrophages have been shown to be capable of releasing PGE_2 in the presence of potent stimulators, e.g., endotoxic lipopolysaccharide (21). The effectiveness in the stimulation of PGE_2 biosynthesis of approximately twofold over basal values in response AVP (500 pM) and its analogues oxytocin (~1¼-fold) and dDAVP (~0.5-fold) is comparable with that obtained from studies with rat medullary interstitial cells (5). The rank order in the stimulation of PGE_2 biosynthesis by mononuclear phagocytes in response to the polypeptides appears to be the same as that for rat medullary interstitial cells (AVP > oxytocin > dDAVP), corresponding with their rank order of pressor activities. The results obtained from radiometric TLC experiments with the cells incubated with [^{14}C]AA and with mononuclear phagocytes labeled overnight with [^{3}H]AA, demonstrated PGE_2 to be the only detectable prostaglandin produced by the cells. The product identification of PGE_2 was further substantiated by the use of different radioimmunoassays for TxB_2 and $PGF_{2\alpha}$ that indicated the lack of significant amounts of PGs other than PGE_2. An additional proof for the stimulatory effect of AVP on PGE biosynthesis was the blockade of PGE_2 release after preincubation of the cells with cyclo VAVP, a synthetic nonpressor substance, and specific nondiuretic antagonist of vasopressin's pressor activity (15).

While high concentrations of PGE_2 that cannot be achieved under in vivo conditions led to a moderate increase in intracellular cyclic AMP content, low concentrations of PGE_2 that can be released by the cells in response to AVP inhibited the AVP-dependent raise in cyclic AMP. This finding agrees with the observation of Omachi et al. (3) and Beck et al. (4), who demonstrated a reduced response in the accumulation of cyclic AMP to AVP in epithelial cells of the bladder and in slices of renal medulla by low concentrations of PGE_2. The time at which PGE_2 is added in relation to AVP thereby appears to be critical. For example, those concentrations of PGE_2 that cause no significant elevation in cytoplasmic cyclic AMP levels must be added before AVP. If, however, PGE_2 is added either simultaneously or after AVP, it no longer has an effect on the ability of AVP to stimulate cyclic AMP metabolism of monocytes. This indicates that those steps in the activity of AVP that are sensitive to effects of cyclic AMP, are the earliest steps involved in the sequence, whereas there are later steps or components involved in the action of AVP that are no longer sensitive to the effect of cyclic AMP. The finding that indomethacin treatment enhanced the AVP-mediated raise in cyto-

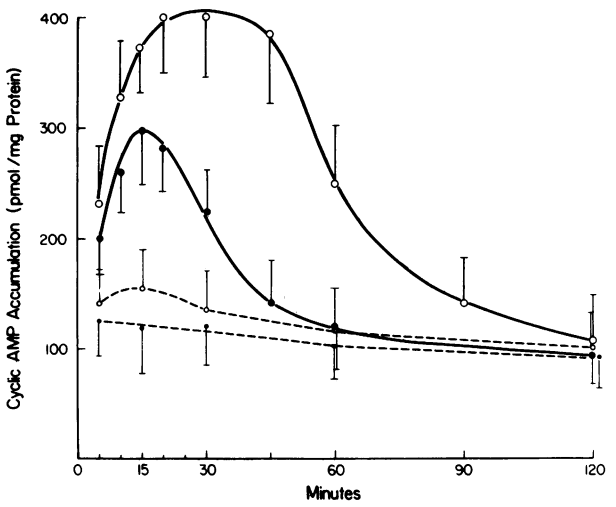

FIGURE 6 Stimulation of cyclic AMP accumulation by AVP (500 pM) in the presence and absence of indomethacin (1 μg/ml), O —— O and ● —— ●, respectively. Cells (8×10^6/ml were preincubated for 30 min at 37°C in the presence and in the absence of indomethacin before the addition of AVP (- - -). The incubation was then continued for the indicated times. Cyclic AMP content in the cells was determined in the presence of IBMX (0.1 mM). The data are expressed in milligrams of cell protein per milliliter and are the mean±SD of four independent experiments.

plasmic cyclic AMP level of mononuclear phagocytes provides further support that endogenous PGE interfere with cellular actions of AVP to generate cyclic AMP. This observation corresponds with a report by Lum et al. (22) who demonstrated the activity of indomethacin to potentiate the renal medullary cyclic AMP response to AVP in vivo.

The molecular basis for the inhibitory effect of PGE_2 on AVP-induced elevation of intracellular cyclic AMP cannot be attributed to agonist-mediated decrease in AVP receptors or to interference with affinity of the hormone, since [^{3}H]AVP binding and displacement of binding by analogs to the cells were not affected by PGE_2. The evidence suggests that the inhibitory effect of PGE_2 on cyclic AMP accumulation resides at a locus subsequent to hormonal binding to the receptor. Additionally, there is evidence for PGE_2 receptors that are coupled to adenylate cyclase in a mode that stimulates the enzyme, which would explain the stimulatory effect of high concentrations of PGE_2 on cyclic AMP accumulation in the mononuclear phagocytes.

Our studies on vasopressin-prostaglandin interactions at human mononuclear phagocytes show striking similarities with that of renal collecting duct and toad urinary bladder relevant for regulation of water balance, in that AVP stimulates intracellular accumulation of cyclic AMP subsequent to binding of the hormone and stimulates PGE_2 biosynthesis. In addition, low concentrations of PGE_2 that are released by the cells after stimulation by the hormone, inhibit cyclic AMP accumulation, indicating a role for PGE_2 as a negative feedback-modulator of vasopressin's activity. Human mononuclear phagocytes may provide a useful instrument to study the interrelationships of both substances in vitro as indicated by these studies.

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