Arginine Vasopressin Stimulates Human Mesangial Cell Production of Endothelin

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Abstract

Endothelin (ET) is a vasoactive peptide produced by both endothelial and epithelial cells with documented mitogenic action on mesangial cells. The present studies were designed to test the hypothesis that ET is also produced by human mesangial cells (HMC) and that other mitogens such as arginine vasopressin (AVP) and insulin stimulate cellular proliferation, in part, through modulation of endogenous production of this peptide. Studies were conducted on cultured normal HMC between the third and seventh passages. All mitogenesis experiments were carried out in 96-well plates and assessed by tritiated thymidine incorporation into DNA under various concentrations of AVP in the presence and absence of insulin, antiendothelin antisera (ETAS), a MAb against ET-1 (AbET), and a vasopressin-1 receptor antagonist. ET concentrations were measured daily from conditioned medium by a sensitive and specific RIA. ET was present in all concentrations of FCS as well as conditioned medium compared with medium alone. AVP (10^{-6} M) in the presence of insulin increased ET production by quiescent HMC by 261% as well as cellular proliferation by 440% after 48 h incubation. In addition, cells cultured with ETAS or AbET demonstrated a blunted mitogenic response to AVP, a response not observed in cells cultured with ETAS where ET was added. Insulin significantly potentiated the mitogenic effects of AVP as well as media levels of ET, an effect significantly blunted by AbET. We conclude that ET is produced by HMC and its production is affected, in part, by both AVP and insulin. ET may thus serve to modulate the mitogenic effects of AVP on human mesangial cells. (*J. Clin. Invest.* 1991. 87:1158–1164.) Key words: mitogenesis • monoclonal antibody • autocrine • insulin

Introduction

Recent studies demonstrate the presence of mRNA for endothelin as well as production of this vasoactive peptide in cells derived from both ectodermal (epithelial) and endodermal (endothelial) origin (1–3). Furthermore, studies in cells derived from mesoderm (glomerular mesangial cells) demonstrate the existence of two different endothelin receptors as well as the ability of exogenous endothelin to increase mitogenesis of these cells (4–7). The present studies were designed to test the hypothesis that normal human glomerular mesangial cells, in culture, produce endothelin and that other mitogenic peptides such as arginine vasopressin and insulin potentiate this production. Furthermore, these experiments were designed to assess whether endogenously produced endothelin modulates, in part, the mitogenic effects of arginine vasopressin (AVP)[1] and insulin on normal human mesangial cells (HMC).

Methods

Materials. Arginine vasopressin was purchased from CalBiochem-Behring Corp. (La Jolla, CA). Endothelin-1 (ET-1) and [1-(β-mercapto-β,β-cyclopentamethylene propionic acid), 2-(*O*-methyl-Tyr)-Arg] vasopressin (PMP) and antiendothelin antisera (ETAS) were obtained from Peninsula Laboratories (Belmont, CA). ^{125}I-ET-1 was obtained from Amersham Corp. (Arlington Heights, IL). Dulbecco's PBS (DPBS), trypsin, EDTA, and RPMI 1640 medium were all obtained from Gibco Laboratories (Grand Island, NY). Insulin was obtained from Eli Lilly Pharmaceuticals (Indianapolis, IN). FCS was obtained from Irvine Scientific (Irvine, CA). [^{3}H]Thymidine was obtained from New England Nuclear (Boston, MA). Immunobead second antibody reagent was obtained from Bio-Rad Laboratories (Richmond, CA). Mice (BALB/CA/JF1) were purchased from Jackson Laboratories (Bar Harbor, ME).

Solutions. AVP was initially dissolved in a 0.1% BSA and Hepes [*N*-2-hydroxyethyl-piperazine N^1-2-ethane sulfonic acid] solution. AVP was then diluted in RPMI 1640 in the stock Hepes/0.1% BSA solution to the desired concentration on the day of the experiment. PMP was prepared by dissolving the chemical in the Hepes/0.1% BSA solution on the day of each experiment. Collin's Medium consisted of 15 mM KCl, 10 mM $NaHCO_3$, 15 mM KH_2PO_4, 42.5 mM K_2HPO_4, and 150 mM glucose.

Isolation and identification of human mesangial cells. Mesangial cells were isolated from renal cortical tissue obtained following total nephrectomy in patients with carcinoma of the renal pelvis. Kidneys were perfused with and maintained in ice-cold Collin's medium at 4°C for < 12 h. After removal of the medulla from kidney tissue, the glomeruli were isolated from renal cortex via differential sieving as previously described (8, 9). Outgrowths of mesangial cells were subcultured 3–4 wk later in 75-cm^2 flasks containing RPMI 1640 and FCS. Identification of these cells was performed by staining the mesangial cells for specific surface antigens. We used murine MAbs to human vimentin and Factor VIII–related antigen to test for specific surface antigens. In addition, further identification was routinely performed using rabbit IgG directed to vascular smooth muscle myosin and FITC-conjugated mouse anti-rabbit IgG. These studies indicated a staining pattern considered indicative of human mesangial cells (8–10).

Our homogenous cultures consist of stellate or fusiform cells with prominent intracellular fibrillar structures. Purity and identification of these cells were continually assessed on a morphologic basis by both

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1. *Abbreviations used in this paper:* AVP, arginine vasopressin; ET, endothelin; ETAS, antiendothelin antisera; HMC, human mesangial cells.

phase contrast microscopy and electron microscopy for each cell preparation. Cells used for our experiments had undergone three to seven passages after initial seeding. Cells were grown at 37°C in 5% CO_2/95% air. Subcultures of mesangial cells were grown in 75-cm^2 flasks in RPMI-1640 that contained varying concentrations of FCS between 0.5, 10, and 20%, 500 ng/ml penicillin, and 100 ng/ml streptomycin. Insulin, 5 μg/ml, was instilled in 50% of the flasks.

For cell proliferation studies, confluent cultures of human mesangial cells were washed three times with DPBS and then removed from culture flasks by standard trypsin-EDTA digestion. Dissociated cells were centrifuged in serum-containing medium, resuspended in complete growth medium (RPMI 1640, 10% FCS, 5 μg/ml insulin, 10 mM L-glutamine, 500 ng/ml penicillin, and 100 ng/ml streptomycin) and subcultured into 96-well plates. Mesangial cells were plated with a density of 10^3 per well in previously described complete growth media until subconfluent. Cells were then washed in DPBS three times before incubating in medium containing 0.5% FCS and RPMI 1640 for 48 h. Cells were then ready for growth modulation studies.

Determination of cellular proliferation. To determine the effects of AVP on human mesangial cell proliferation, 10 μl of AVP per well were added to obtain the desired concentration. The AVP antagonist PMP [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-(O-methyl-Tyr)Arg] was added as a 10-μl volume to the individual well 1 h before addition of AVP. [^{3}H]Thymidine was added at 1 μCi per well 24 h before harvesting. Cellular proliferation studies were performed 24, 48, and 72 h after addition of AVP. Control wells received only the appropriate vehicle solution. All experiments were performed in both quiescent (0.5% FCS) and proliferative (10% FCS) conditions in the presence and absence of insulin 5 μg/ml. A separate set of experiments were performed under these conditions to assess the interaction of 5 μg/ml insulin on mitogenesis in the absence of endothelin as described below.

To assess the mitogenic effect of AVP [10^{-6} M] in the absence of endothelin, we endeavored to bind endogenously produced endothelin by human mesangial cells using previously described methodology (11). Clozel et al., using conditioned medium from human smooth muscle cells, demonstrated significant binding of endothelin by ETAS (11). We used subconfluent HMCs in 96-well plates (passages 4–6) for proliferation studies on these cells. Proliferation studies were conducted in a manner previously described under both quiescent (0.5% FCS) and proliferative (10% FCS) conditions in the presence and absence of insulin and/or endothelin (10^{-9} M). All studies were performed in triplicate. 50% of the wells received ETAS at a dilution of 1:1,200, a dilution previously shown to inhibit the activity of ET of human smooth muscle cells (11). Previous studies in our laboratory demonstrate that this dilution of antisera inhibits binding of 50 pM of ^{125}I-ET-1 by ~ 85% (data not shown). In addition, parallel growth studies on HMCs were conducted in 96-well plates to assess the degree of inhibition of mitogenesis by the ETAS at this dilution.

Extracts of conditioned media containing 10% FCS from confluent cells were removed and incubated for 20 min with ETAS to a final dilution of 1:1200. The Immunobead second antibody was then added (4.0 mg/ml media) to remove both free and bound ET-1 antiserum present in the media as previously described (11). The suspensions were heated at 37°C for 2 h followed by centrifugation for 7 min at 1,000 *g* (11). The supernatant was removed, lyophilized, and used for competition experiments. Competition experiments with conditioned medium not treated with ETAS were performed in parallel (data not shown).

To further support the findings of these studies, we repeated the above mentioned studies using a MAb against ET-1. A MAb to ET-1 was developed in the following manner: pure endothelin was obtained and conjugated to keyhole limpet hemocyanin as previously described (12–13). The conjugated peptide was mixed in CFA and used for immunization of 5–8-wk-old (BALB/cA/JF1) mice. Immunization, cell fusion (fusion cells are SP/02), cloning by limited dilution, and ascites production were carried out as previously described (13). The endothelin MAb was found to be specific for ET-1 but had a > 50% reactivity with ET-2 and < 30% reactivity with ET-3 as determined by RIA. The antibody detected ET-1 at a dilution of 1:100,000 with a sensitivity of 1 pM/ml. The binding affinity (equilibrium dissociation constant; K_d) for ET-1 as determined by RIA at a dilution of 1:100,000 was 1 nM. The antibody did not cross-react with secretin, vasoactive intestinal peptide, β-endorphin, somatostatin, calcitonin gene–related peptide, and peptide yy. The antibody is stable for more than a year when stored frozen at −80°C. The diluted antibody is stable at 4°C for a period of 4 wk.

RIA studies of MAb to ET-1 were carried out following dilution to an appropriate concentration (1:100,000) with 10 mM phosphate buffer, pH 7.4, containing 0.1% BSA. To construct the standard curve, aliquots (50 ml) of the diluted antibody were incubated at 0°C for 16 h with 25 pmol of ^{125}I-endothelin (50 ml, 15,000 cpm) in the absence (control) or in the presence of unlabeled endothelin (100 pM to 100 nM) to a final volume of 200 ml of phosphate buffer. To determine ET in unknown samples, aliquots of the unknown sample (100 ml) were mixed with 50 ml of the antibody and 50 ml of the radiolabeled ET and incubated as above. To remove the unbound radiolabeled ET, samples were treated with dextran-coated charcoal suspension (0.5% acid washed activated charcoal Norit A, 0.05% dextran 70,000 prepared in 50 mM Tris buffer, pH 7.4, centrifuged, and resuspended in 10 mM phosphate buffer). Aliquots of the dextran-coated charcoal were then added to each test tube and incubated at 4°C for 20 min with intermittent vortexing (13). The suspension was then centrifuged at 4°C for 15 min and an aliquot of the supernatant was removed and counted. The radioactivity is corrected for sample volume and isotope decay.

To assess the effect of a MAb against ET-1 and the subsequent effect on AVP and insulin-induced mitogenesis, we prepared cells for proliferation studies as previously mentioned and used 10 ml of a dilution of 1:50,000 of a MAb to ET-1 in 96-well plates under both quiescent (0.5% FCS) and proliferative (10% FCS) conditions in the presence and absence of AVP (10^{-6} M) as well as insulin. Studies were performed at 48 h in a manner similar to that previously described. All studies were performed in triplicate. DNA replication studies of HMC, [^{3}H]-thymidine incorporation into DNA was used. Mesangial cells were detached from plates with trypsin/EDTA and [^{3}H]thymidine-containing DNA was collected on glass fibrostrips using an automated cell harvester (Pharmacia LKB, Piscataway, NJ). [^{3}H]Thymidine content was determined using a BetaMax liquid scintillation counter at 5 min per sample. All conditions were performed in triplicate. Cell counts were routinely conducted in all conditions to ascertain HMC replication, microscopically. The number of viable cells was determined by first washing the cells with DPBS three times, then incubating them in 50 μl of trypsin for 30 min at 37°C, which results in complete detachment of HMC. The detached cells were resuspended with equal volumes of 0.4% trypan blue and growth medium and transferred to a counting chamber where the number of viable cells was counted using inverted phase contrast microscopy.

Determination of mesangial cell endothelin production. ET was measured in conditioned medium from both quiescent (0.5% FCS) and proliferative (10% FCS) conditions in the presence and absence of insulin as well as under various concentrations of arginine vasopressin. Endothelin assays were performed immediately after each group of experiments.

HMC were plated in 75-cm^2 flasks and allowed to achieve confluency. Cells were then washed three times with DPBS, and basal medium containing 0.5% FCS was added. After 48 h the medium was aspirated from each flask and all cells were washed with DPBS. Then flasks received either basal medium (0.5% FCS) or growth medium (10% FCS); only half of each set of flasks contained insulin 5 μg/ml. ET was then measured in conditioned medium at 24, 48, and 72 h. This procedure was repeated in a separate set of experiments, each in triplicate, where either 10^{-10} M, 10^{-8} M, or 10^{-6} M AVP was added to each flask in the presence or absence of insulin. Similar determinations of ET were carried out at 24, 48, and 72 h in conditioned medium of cells that were used in studies using ETAS that did not receive the antiserum. All endothelin values in the text are corrected for ET present in the accompanying media. ET was measured using a specific and sensitive RIA which has been previously described (14). The level of detect-

ability of this assay was 0.5 pg/ml with a range of 0.5–400 pg/ml. The interassay and intraassay variations were 7.5±1.2 and 2.1±1.5%, respectively.

In addition, experiments to determine the presence of endothelin in FCS were performed examining various concentrations of FCS (0.5, 5, 10, and 20%). ET was also measured in RPMI 1640 alone as control.

Statistics. Data were analyzed using both analysis of variance with subgroup analysis and Student's *t* test for paired and unpaired groups, where applicable. All data are expressed as mean±SEM, unless otherwise noted.

Results

Endothelin production. ET levels were measured in various concentrations of FCS and compared with medium alone (Fig. 1). Furthermore, ET was present at higher concentrations in conditioned medium after 48 h incubation with mesangial cells compared with FCS during the same latency period (Fig. 1). Thus, even when corrected for concentrations of ET in FCS, quiescent HMC produce on average 2.2±0.4 pg/ml of ET at 48 h in the presence of insulin. Moreover, ET production appears to plateau at 48 h, a trend that correlates with cellular proliferation (Fig. 2).

Insulin at a concentration of 5 μg/ml did not significantly change ET concentration in quiescent cells (Fig. 2). However, when insulin was added to either proliferative or quiescent cells in the presence of varying concentrations of AVP, there was a significant increase in ET concentrations above that seen with AVP alone (Figs. 2–4).

AVP alone in ascending concentrations increased ET production on proliferative as well as quiescent mesangial cells in a dose-dependent fashion, an effect that correlated with its effect on mitogenesis and cell counts (Figs. 2–4). Calculation of ET production per cell demonstrated a 76% increase by proliferative cells (10% FCS) in the presence of AVP (10^{-6} M) plus

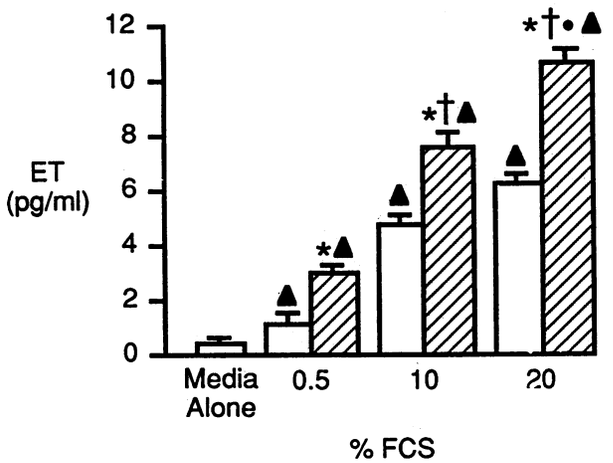

Figure 1. The comparative concentrations of ET in varying concentrations of FCS alone versus conditioned medium from HMC after 48 h. Insulin (5 μg/ml) is present in all conditions tested. The white boxes represent FCS alone, while the hatched boxes represent conditioned medium. (*) $P < 0.05$ compared with FCS alone; (+) $P < 0.05$ compared with conditioned medium (0.5% FCS); (·) $P < 0.05$ compared with conditioned medium (10% FCS); (▲) $P < 0.05$ compared with media alone. Please note that concentration of ET in conditioned media were corrected for presence of ET in FCS.

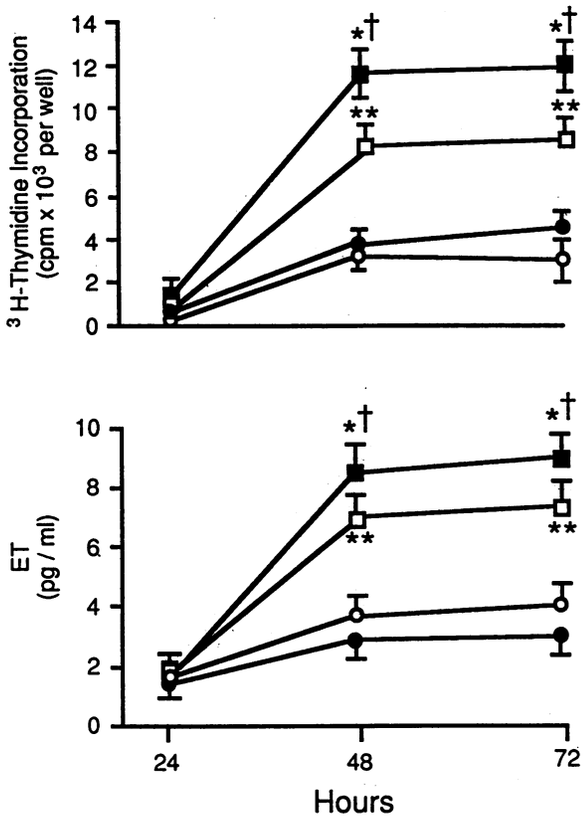

Figure 2. The effect of AVP in the presence or absence of insulin on the time course of mitogenesis and ET production by quiescent (0.5% FCS) HMC. Open circles represent cells without insulin or AVP; closed circles represent cells with insulin and no AVP. Open squares represent cells with AVP (10^{-6} M) without insulin. Closed squares represent cells with insulin and AVP (10^{-6} M). (*) $P < 0.05$ compared with cells without insulin with AVP; (+) $P < 0.05$ compared with cells with insulin and without AVP; (**) $P < 0.05$ compared with cells without AVP or insulin.

insulin compared with vehicle plus insulin (0.00050 pg/cell, vehicle vs. 0.00088 pg/cell, AVP; $P < 0.01$) and a 25% increase by quiescent cells (0.00097±0.00003 pg/cell vehicle vs. 0.0012±0.00005 pg/cell AVP; $P < 0.0002$). Furthermore, evaluation of ET production per cell in two different culture milieus, 0.05% FCS and 20% FCS, both in the presence of insulin and absence of AVP, did not significantly differ in ET concentration per cell (cell counts not shown) (0.00091±0.00004 pg/cell, quiescent vs. 0.00085±0.00005 proliferative).

Addition of a selective V_1 receptor antagonist, PMP, 1 h before addition of AVP markedly attenuated the rise in ET concentration in conditioned medium as well as mitogenesis (Fig. 5 and Table I). This inhibitory effect of PMP was overcome by simultaneous addition of ET (10^{-9} M) to PMP (Table I). Furthermore, while insulin significantly increased both ET concentration in conditioned medium and mitogenic activity of AVP, it failed to increase mitogenesis of ET concentration in conditioned medium in the presence of PMP (Fig. 5).

[^{3}H]Thymidine incorporation and mitogenesis. AVP increased mesangial cell proliferation in a dose-dependent fashion (Figs. 3 and 4; Table I). This effect was further potentiated

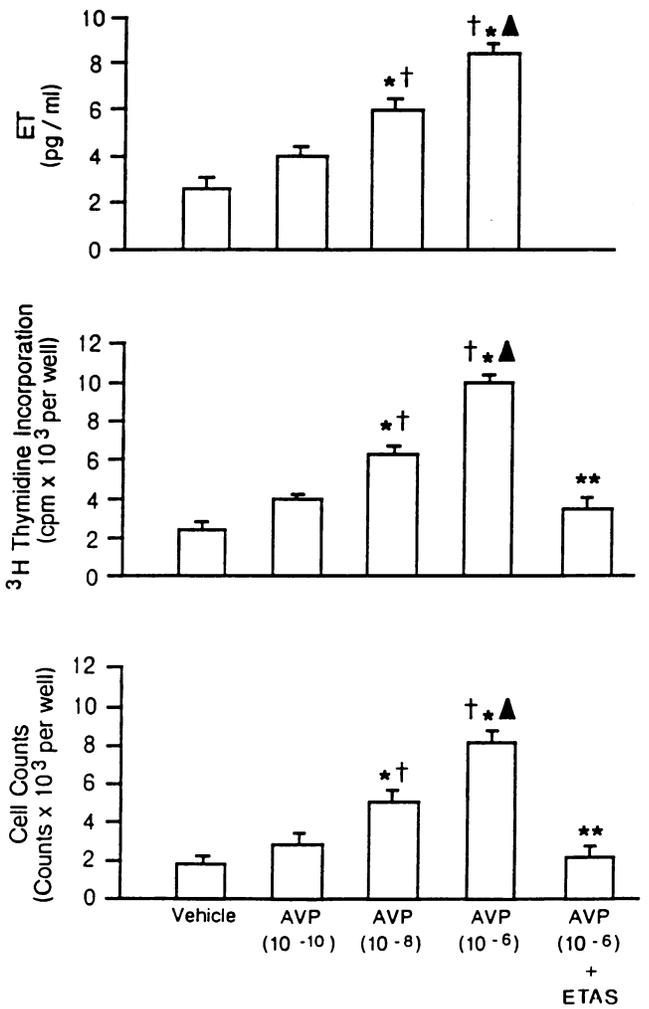

Figure 3. The effects of varying concentrations of AVP on ET levels in conditioned medium and cellular proliferation in the presence of insulin and ETAS on quiescent (0.5% FCS) HMC after 48 h. (*) $P < 0.05$ compared with vehicle; (+) $P < 0.05$ compared with 10^{-10} M AVP; (▲) $P < 0.05$ compared with 10^{-8} M AVP; (**) $P < 0.05$ compared with 10^{-6} M AVP.

by insulin (Figs. 2, 5–7). However, this stimulatory effect of insulin on mitogenesis was markedly attenuated in the presence of either ETAS, a MAb against ET-1, or the V_1 receptor antagonist, PMP (Table I and Figs. 3–7). Cell counts correlated with this blunted effect in cell mitogenesis (Figs. 3–7), with no significant differences in cell viability between the different conditions tested (data not shown). Furthermore, the inhibitory effect of ETAS, AbET, or PMP could not be overcome by addition of insulin to AVP (10^{-6} M) (Figs. 3–7). However, addition of endothelin (10^{-9} M) in the presence of PMP and insulin resulted in a significant increase in mitogenesis, an effect that approximated vasopressin alone (Table I). This effect on mitogenesis was not seen in the absence of insulin (7.3±0.4 cpm × 10^3/well, AVP [10^{-6} M] alone, compared with 3.5±0.3 cpm × 10^3/well, AVP [10^{-6} M] + PMP [10^{-6} M] + ET [10^{-9} M]). Presence of the MAb against ET-1 demonstrates a marked attenuation in mitogenesis of these cells, an effect potentiated by the presence of insulin (Figs. 6–7).

Discussion

The results of the present experiments demonstrate that (*a*) ET is produced by HMC and is present in FCS; (*b*) AVP increases HMC production of ET in a dose-dependent fashion; (*c*) inhibition of ET with ETAS or a MAb against ET-1 partially blunts the mitogenic effects of arginine vasopressin on HMC; and finally, (*d*) insulin potentiates the mitogenic effects of AVP on HMC; however, it also appears to augment the antimitogenic effects of ETAS as well as a MAb against ET-1.

Our studies confirm previous observations that AVP increases mitogenesis of mesangial cells in a dose-dependent manner and that insulin potentiates this effect (15). We further support the finding that insulin augments for the mitogenic effect of ET on these cells (7). Furthermore, the dose and time course over which this occurred is similar to previous reports (15). In addition, our data confirm previous reports that selective inhibition of V_1 receptors attenuates the mitogenic response of vasopressin in the presence and absence of insulin (15, 16).

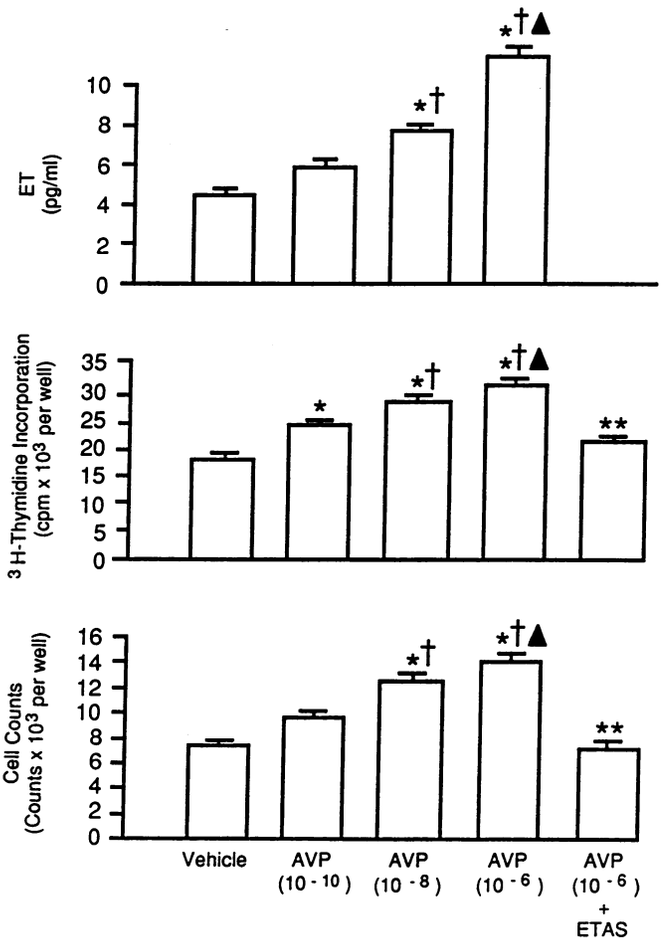

Figure 4. The effects of varying concentrations of AVP on ET levels in conditioned medium and cellular proliferation in the presence of insulin and ETAS on proliferative (10% FCS) mesangial cells after 48 h. (*) $P < 0.05$ compared with vehicle; (+) $P < 0.05$ compared with 10^{-10} M AVP; (▲) $P < 0.05$ compared with 10^{-8} M AVP; (**) $P < 0.05$ compared with 10^{-6} M AVP.

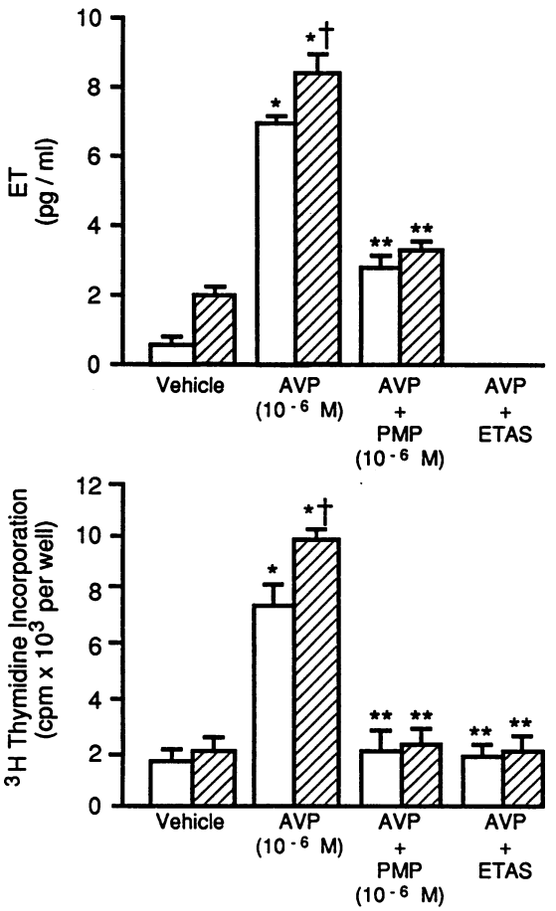

Figure 5. The effects of AVP with either ETAS or PMP in the presence or absence of insulin on cellular mitogenesis and ET concentration in conditioned medium from quiescent (0.5% FCS) HMC at 48 h. Open boxes represent studies without insulin, hatched boxes represent studies with insulin. (*) $P < 0.05$ compared with vehicle; (+) $P < 0.05$ compared with no insulin; (**) $P < 0.05$ compared with to AVP (10^{-6} M).

The interrelationship between AVP and cellular production of ET, however, has not been previously described in HMC. We present evidence that AVP increases ET production by HMC. We also demonstrate that binding of ET, produced by HMC, with either ETAS or a MAb directed against ET-1 significantly attenuates the mitogenic effects of AVP. Further evidence to support the notion that AVP modulates its mitogenic effect through stimulation of ET is supported by the observation that addition of ET-1 to these cells, pretreated with a selective vasopressin-1 receptor antagonist, results in increased cell growth and mitogenic activity.

One possible explanation for the observation that ET concentration increases after administration of AVP is that ET concentration in the media is a reflection of increased cell number. However, when ET concentration is evaluated per cell, there is clearly a greater increase in the cells treated with AVP compared with vehicle, thus supporting the notion that ET concentration is truly elevated after AVP administration. Second, a comparison of ET concentration per cell in both quiescent (0.5% FCS) and proliferative (20% FCS) culture milieus in the presence of insulin and absence of AVP does not demonstrate an increased ET concentration per cell. Taken together, these data support the concept that ET production is increased by AVP and that AVP stimulates mitogenesis of HMC, in part by stimulating endogenous ET production.

The sole mitogenic effect of AVP on mesangial cells does not totally depend on its stimulation of ET production by these cells. We demonstrate that addition of ET to cells previously treated with both AVP and a vasopressin receptor antagonist only approximated (~ 72%) the mitogenic effect of AVP alone. A number of possible explanations may account for this observation. First, mesangial cells are known to contain mRNA of various growth factors. These include: platelet-derived growth factor, IGF-I, transformation growth factor β, IL-1, etc. These factors have been shown to modulate the mitogenic response of many growth factors and all but transformation growth factor β increase cellular proliferation (17–19). It is possible that some or all of these growth factors participate in the mitogenic action of AVP, thus accounting for only a partial mitogenic response when ET was added to the culture milieu, although no data supporting this contention are currently available. Second, both AVP and ET may stimulate growth through a calcium-dependent protein kinase C mechanism (20–21). However, the relative stimulatory effect of ET on cytosolic free calcium is much less than that of AVP (21). This disparity of response may also contribute to the discrepancy in the mitogenic effect between these two peptides.

Insulin is known to be a potent mitogen in certain tumor cell lines through its binding to a specific tyrosine kinase receptor and through activation of protein kinase (22, 23). The mitogenic effects of insulin on mesangial cells are known to result, in part, from stimulation of IGF-I receptors rather than insulin receptors on these cells (24). In addition, stimulation of IGF-I receptors has been shown to potentiate the effects of other vasoactive hormones (25). Previous studies also show that insu-

Table I. The Effects of the V_1 Antagonist PMP and Endothelin on AVP (10^{-6} M) Induced Increases in Mitogenesis and Presence of Endothelin in Conditioned Medium after 48 h of Incubation

	[^{3}H]Thymidine incorporation into DNA	ET
	cpm × 10^3/well	*pg/ml*
Baseline	2.3±0.4	2.1±0.3
PMP (10^{-6} M) alone	2.4±0.3	2.6±0.4
AVP (10^{-6} M) alone	8.4±0.3*	10.7±0.5*
AVP + PMP (10^{-8} M)	4.7±0.2*	5.3±0.4*
AVP + PMP (10^{-6} M)	2.9±0.3‡	3.2±0.3‡
AVP + PMP (10^{-6} M) + ET (10^{-9} M)	6.2±0.3§	

Values are expressed as mean±SEM (each condition was repeated in quadruplicate). All above experiments were performed in the presence of insulin in quiescent cells (0.5% FCS).
* $P < 0.05$ compared with baseline.
‡ $P < 0.05$ compared with AVP alone.
§ $P < 0.05$ compared with AVP + PMP (10^{-6} M). Data analysis was performed.

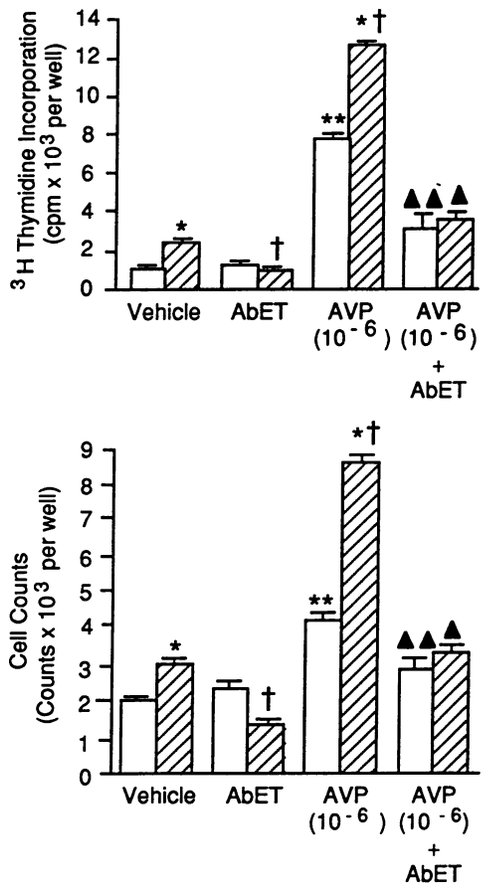

Figure 6. The effect of AVP (10^{-6} M) on mitogenesis in quiescent (0.5% FCS) HMC in the presence or absence of a MAb against ET-1 or insulin at 48 h. Open boxes without insulin; hatched boxes with insulin. (*) $P < 0.05$ compared with corresponding group of cells without insulin; (+) $P < 0.05$ compared with vehicle cells with insulin; (**) compared with vehicle without insulin; (▲) compared with AVP (10^{-6} M) with insulin; (▲▲) compared with AVP alone without insulin.

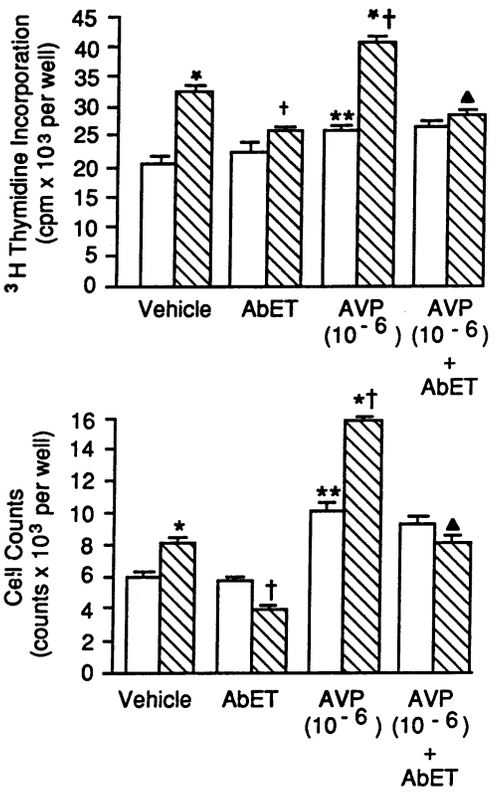

Figure 7. The effect of AVP (10^{-6} M) on mitogenesis in proliferative (10% FCS) HMC in the presence or absence of a MAb against ET-1 or insulin at 48 h. Open boxes without insulin; hatched boxes with insulin. (*) $P < 0.05$ compared with corresponding group of cells without insulin; (+) $P < 0.05$ compared with vehicle cells with insulin; (**) compared with vehicle without insulin; (▲) compared with AVP (10^{-6} M) with insulin.

lin, at a dose used in our study, potentiates the mitogenic effects of various vasoactive peptides on mesangial cells, although insulin by itself is a relatively weak mitogen in comparison to these peptides (15, 26–27). Furthermore, the action of various mitogenic peptides such as AVP or angiotensin II on mesangial cells is markedly blunted or absent if insulin is not present in the culture milieu (15, 28, 29). Our data support these findings in that insulin potentiates the mitogenic effect of AVP in HMC. Furthermore, insulin, in the absence of AVP, had no significant effect on endogenous ET production, an effect that was markedly altered in the presence of AVP. Addition of insulin to the culture milieu also potentiated the antimitogenic effects of ETAS as well as the MAb against ET-1. This effect of insulin, however, still needs to be fully elucidated.

We conclude that the vasoactive peptide ET modulates, in part, the mitogenic response of AVP on HMC. We further conclude that insulin augments this interaction. Further experiments that explore the interactions between these two peptides may help elucidate the behavior of mesangial cells under other mitogenic stimuli such as angiotensin II, platelet-derived growth factor, and the like.

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