Available online at www.sciencedirect.com
SCIENCE DIRECT
European Journal of Pharmacology 538 (2006) 32–38
www.elsevier.com/locate/ejphar

Effects of YM218, a nonpeptide vasopressin V_{1A} receptor-selective antagonist, on vasopressin-induced growth responses in human mesangial cells

Atsuo Tahara *, Junko Tsukada, Yuichi Tomura, Kazuhiro Momose, Takeshi Suzuki, Takeyuki Yatsu, Masayuki Shibasaki

Institute for Drug Discovery Research, Astellas Pharma Inc., 5-2-3, Toukoudai, Tsukuba, Ibaraki 300-2698, Japan

Received 10 March 2006; received in revised form 21 March 2006; accepted 27 March 2006
Available online 1 April 2006

Abstract

Mesangial cells are centrally-located glomerular pericytes with contractile, endocrine, and immunity-regulating functions. These cells are thought to maintain normal glomerular function, since mesangial cell proliferation and extracellular matrix formation are hallmarks of chronic glomerular disease. Vasopressin causes mesangial cell contraction, proliferation and hypertrophy. Consequently, the effects of YM218, a potent, nonpeptide vasopressin V_{1A} receptor-selective antagonist, on the growth responses of human mesangial cells to vasopressin were investigated. YM218 showed high affinity for vasopressin V_{1A} receptors, exhibiting a K_i value of 0.18 nM. Vasopressin concentration-dependently increased intracellular Ca^{2+} levels and induced hyperplasia and hypertrophy in cultured mesangial cells, YM218 potently inhibited these vasopressin-induced responses. These results clearly show that YM218 has both strong affinity for human mesangial cell vasopressin V_{1A} receptors and great potency in inhibiting the vasopressin-induced growth responses of mesangial cells controlled by the vasopressin V_{1A} receptors. The hyperplasia and hypertrophy of mesangial cells in vitro caused by vasopressin indicate its possible in vivo role in glomerular disease pathogenesis. Therefore, YM218 is a potent pharmacologic probe to investigate the physiologic and pathophysiologic roles of vasopressin in the development of renal disease.

Keywords: YM218; Vasopressin V_{1A} receptor; Nonpeptide antagonist; Mesangial cell

1. Introduction

Mesangial cells are thought to regulate both glomerular microcirculation and inflammatory response to glomerular damage (Kashgarian and Sterzel, 1992). Additionally, contraction, hyperplasia and hypertrophy of glomerular mesangial cells are associated with the appearance of proteinuria, glomerulosclerosis, and progressive renal failure (Striker et al., 1989). Mesangial cells have receptors that respond to specific vasoactive peptides such as angiotensin II, endothelin-1, and vasopressin (Rebibou et al., 1992; Chansel et al., 1992; Thibonnier et al., 1993); contraction, hyperplasia, hypertrophy, and other physiologic responses are mediated by these receptors (Orth et al., 1995; Simonson et al., 1991; Bakris et al., 1991). These circulating vasoactive hormones easily penetrate the highly permeable mesangial area to exert their effects on glomerular function (Schlondorff, 1987). Among them, vasopressin has been shown to be a potent inducer of mesangial cell proliferation (Schulze-Lohoff et al., 1993). The physiologic actions of vasopressin on mesangial cells are mediated through the vasopressin V_{1A} receptor subtype (Ganz et al., 1988). Recent experiments, making use of cultured cells and other recently developed analytic techniques, have established that vasopressin activates mesangial cells through the phospholipase C-mediated breakdown of phosphoinositides; this process generates the second messengers inositol triphosphate and diacylglycerol (Ganz et al., 1988; Mene et al., 1989). Inositol triphosphate causes increased intracellular concentrations of Ca^{2+} ($[Ca^{2+}]_i$) by triggering the release of intracellular calcium stores and by extracellular entry. Simultaneously diacylglycerol activates protein kinase C, which takes on a membrane-associated form that controls the functions of numerous target proteins and enzymes; this control is exerted either independently

* Corresponding author. Tel.: +81 29 847 8911; fax: +81 29 847 1536.
E-mail address: atsuo.tahara@jp.astellas.com (A. Tahara).

0014-2999/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2006.03.055

or in synergy with $[Ca^{2+}]_i$. This pathway ultimately leads to mesangial cell contraction, hyperplasia, hypertrophy in response to vasopressin.

Several lines of evidence indicate the possibility of involvement of vasopressin in renal disease. Chronic inhibition of the vasopressin V_{1A} receptor using OPC-21268, a vasopressin V_{1A} receptor-selective antagonist, suppressed mRNA expressions of proliferating cell nuclear antigen but had no effect on renal histology (Otsuka et al., 1997) in salt-loaded spontaneously hypertensive rats (Otsuka et al., 1997) and improved glomerular sclerosis in spontaneously hypercholesterolemic rats (Kurihara et al., 1996). These results suggest that vasopressin may play an important role through vasopressin V_{1A} receptors in the development of glomerular diseases and vasopressin V_{1A} receptor-selective antagonists may be beneficial in the prevention of renal disease. Previously, several orally effective, receptor-subtype-selective nonpeptide AVP receptor antagonists have been discovered, namely the V_{1A} receptor-selective antagonist SR 49059 (Serradeil-Le Gal et al., 1993), the V_2 receptor-selective antagonist SR 121463A (Serradeil-Le Gal et al., 1996), the V_{1B} receptor-selective antagonist SSR 149415 (Serradeil-Le Gal et al., 2002) and the V_{1A}/V_2 receptor antagonists conivaptan (YM087) and YM471 (Tahara et al., 1997; Tsukada et al., 2002). Recently, an orally effective, nonpeptide V_{1A} receptor-selective antagonist, YM218, was discovered (Tahara et al., 2005). In the present study, the effects of vasopressin receptor antagonists, including YM218, on vasopressin-induced growth responses in cultured human mesangial cells were investigated to assess the role vasopressin may play in the pathogenesis of renal disease.

2. Methods

2.1. Materials

[^{3}H]vasopressin with a specific activity of 80 Ci/mmol was obtained from PerkinElmer, Inc. (Boston, MA, USA). Vasopressin and oxytocin were obtained from Peptide Institute Inc. (Osaka, Japan). d(CH_2)$_5$Tyr(Me)vasopressin ([β-mercapto-β,β-cyclopentamethylene-propionyl^1,*O*-Me-Tyr^2,Arg^8]-vasopressin) and dDAVP ([deamino-Cys^1,*D*-Arg^8]-vasopressin) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). YM218 ((*Z*)-4′-{4,4-difluoro-5-[2-oxo-2-(4-piperidino-piperidino)ethylidene]-2,3,4,5-tetrahydro-1*H*-1-benzoazepine-1-carbonyl}-2-methyl-3-furanilide hemifumarate), SR 49059 ((2*S*) 1-[(2*R* 3*S*)-(5-chloro-3-(2-chlorophenyl)-1-(3,4-dimethoxybenzenesulfonyl)-3-hydroxy-2,3-dihydro-1*H*-indole-2-carbonyl]-pyrrolidine-2-carboxamide) and SR 121463A (1-[4-(*N*-tert-butyl-carbamoyl)-2-methoxybenzenesulfonyl]-5-ethoxy-3-spiro-[4-(2-morpholinoethoxy) cyclohexane]indol-2-one; equatorial isomer) were synthesized by Astellas Pharma Inc. (Ibaraki, Japan). These nonpeptide antagonists were initially dissolved in dimethyl sulfoxide at a concentration of 10^{-2} M and diluted to the desired concentration with the assay buffer. The final concentration of dimethyl sulfoxide in the assay buffer did not exceed 1%, an amount that does not affect specific [^{3}H]vasopressin binding. Fura 2-AM was purchased from Dojindo Laboratories (Kumamoto, Japan).

2.2. Cell culture

Human mesangial cells imported from Clonetics (San Diego, CA, USA) were purchased from IWAKI (Tokyo, Japan). The cells were grown at 37 °C in Mesangial Basic Medium (Clonetics) supplemented with 5% fetal calf serum and antibiotics (GA-1000) in a humidified atmosphere of 5% CO_2 in air. The cultures were subcultured by treatment with a 0.05% trypsin-0.53 mM EDTA solution in 150 cm^2 culture dishes. Cells at passages 4–10 were used in this study and were confirmed to be mesangial cells by their histochemical reactions with anti-cytokeratin, fibronectin and factor VIII antibodies.

2.3. [^{3}H]vasopressin binding assays

Membrane preparations were isolated as follows. Confluent mesangial cells were washed with phosphate-buffered saline; harvested into ice-cold 50 mM Tris–HCl, pH 7.4, containing 250 mM sucrose and 10 mM $MgCl_2$; and homogenized in a Polytron (Kinematica; Lucerne, Switzerland) at 4 °C. After centrifugation at 2000 ×*g* for 10 min at 4 °C, the supernatant was centrifuged at 40 000× *g* for 20 min at 4 °C. The resulting pellet was resuspended in 50 mM Tris–HCl, pH 7.4, containing 10 mM $MgCl_2$, and the resuspended membrane preparations were stored in small aliquots at −80 °C until use. Protein concentration was determined by the Coomassie blue method (Bio-Rad; Hercules, CA, USA), using bovine serum albumin as a standard. For saturation binding studies, membrane preparations were incubated with various concentrations of [^{3}H]vasopressin in the range from 0.15 to 3.0 nM. For competition studies, [^{3}H]vasopressin (0.5–1.0 nM) was added to membrane preparations, which were then incubated with various concentrations of compounds in 250 μl of assay buffer containing 50 mM Tris–HCl, pH 7.4, 10 mM $MgCl_2$ and 0.1% bovine serum albumin. All binding reactions were started by adding the membrane preparations to the reaction mixture. After incubation at 25 °C for 60 min, the reactions were stopped by adding 3 ml of ice-cold Tris buffer (50 mM Tris–HCl, pH 7.4, and 10 mM $MgCl_2$) followed immediately by rapid filtration through a 96-well GF/B UniFilter Plate using a MicroMate Cell Harvester (Packard Instrument Company; Meriden, CT, USA). The filters were rinsed twice and the radioactivity retained on the filters was counted with a TopCount Microplate Scintillation Counter (Packard Instrument Company). Nonspecific binding was determined using 1 μM unlabeled vasopressin. The concentration of test compound that caused 50% inhibition (IC_{50}) of [^{3}H]vasopressin specific binding was determined by regression analysis of displacement curves. The inhibition constant (K_i) was calculated as previously reported (Tahara et al., 1997). Data were analyzed using the GraphPad PRISM (GraphPAD Software, Inc.; San Diego, CA, USA).

2.4. Measurement of $[Ca^{2+}]_i$

Serum-deprived monolayer cultures of mesangial cells were grown for 1 day on plastic cover slips (Sumitomo Bakelite Co.; Akita, Japan) before assay. Cell monolayers (2×10^5 cells/cm^2) were loaded with fura 2-AM (4 μM/cover slip) in Krebs–

Henseleit–HEPES buffer (containing 130 mM NaCl, 5 mM KCl, 1.25 mM $CaCl_2$, 0.8 mM $MgSO_4$, 5.5 mM glucose, 20 mM HEPES and 0.1% bovine serum albumin, pH 7.4) for 30 min at 37 °C. They were then washed, transferred to fura 2-free Krebs–Henseleit–HEPES buffer and incubated for an additional 30 min at 37 °C. The cover slips were placed into quartz cuvettes containing 2 ml Krebs–Henseleit–HEPES buffer maintained at 37 °C while continuously stirred. Fluorescence was recorded using a CAF-110 spectrofluorometer (Japan Spectrometer Co.; Tokyo, Japan) at 340/380 nm excitation and 500 nm emission wavelengths. $[Ca^{2+}]_i$ was calculated from the fluorescence signals as previously reported (Tahara et al., 1997).

2.5. Determination of cell number and total protein content

Mesangial cells were seeded into 48-well culture plates at 50% confluence, washed with phosphate-buffered saline and incubated for 48 h in culture medium supplemented with 0.5% fetal calf serum. The cultures were then incubated for 72 h in medium supplemented with 0.5% fetal calf serum and 0.1% bovine serum albumin containing vehicle alone or various concentrations of vasopressin, antagonists, or both. To determine the number of cells per culture well, the cells were treated with AlamarBlue (Iwaki; Tokyo, Japan; Ahmed et al., 1994) during the last 3 h of incubation. The absorbance of each well at 570 and 600 nm was then measured with a SPECTRAmax 250 microplate spectrophotometer (Molecular Devices; Sunnyvale, CA, USA). Control experiments showed a linear relationship between absorbance and cell number up to a cell density of 100 000 cells/well. To determine the total protein content of the cells in each culture well, the cell layers were then washed three times with phosphate-buffered saline and scraped from the plates. The suspensions were homogenized and assayed for protein content.

2.6. Statistical analysis

Results are expressed as the mean ± S.E.M. or the mean with 95% confidence limits. All experiments were repeated at least three times, and comparable results were obtained. The EC_{50} and IC_{50} values were estimated from concentration–response curves generated by the nonlinear regression program GraphPad PRISM.

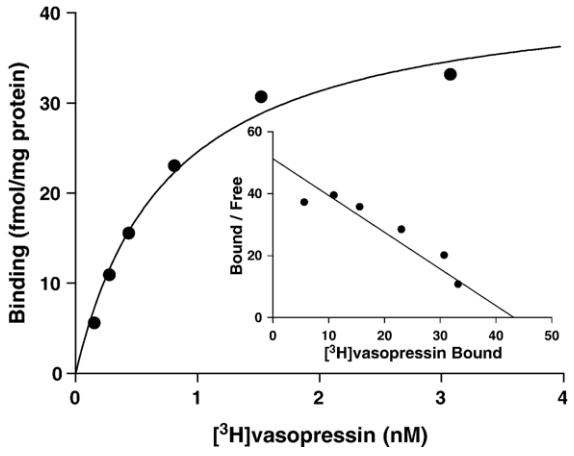

Fig. 1. Plot showing the saturation equilibrium specific binding of [^{3}H]vasopressin to human mesangial cell plasma membranes. Inset, Scatchard linear transformation of the data. Results are representative data from four individual experiments.

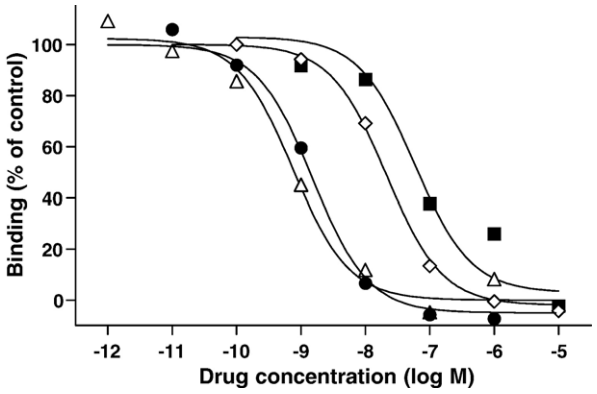

Fig. 2. [^{3}H]vasopressin binding to human mesangial cell plasma membranes is inhibited by peptide vasopressin receptor agonists and antagonists. The unlabeled compounds added to the binding assay were: ●, AVP; ◊, oxytocin; △, d(CH$_2$)$_5$Tyr(Me)vasopressin; ■, dDAVP. Specific binding of [^{3}H]vasopressin is expressed as a percentage of the control binding. Results represent data from three to four independent determinations.

3. Results

3.1. [^{3}H]vasopressin binding to human mesangial cell plasma membranes

Saturation experiments using [^{3}H]vasopressin and human mesangial cell plasma membrane preparations revealed that vasopressin binding was specific and saturable. Scatchard analysis of data revealed a linear plot consistent with the presence of a single class of high-affinity binding sites (Fig. 1). The apparent equilibrium dissociation constant (K_d) and the maximal binding capacity (B_{max}) were 0.81 ± 0.06 nM and 53.8 ± 7.0 fmol/mg protein, respectively. Competition experiments showed that [^{3}H]vasopressin binding was potently and

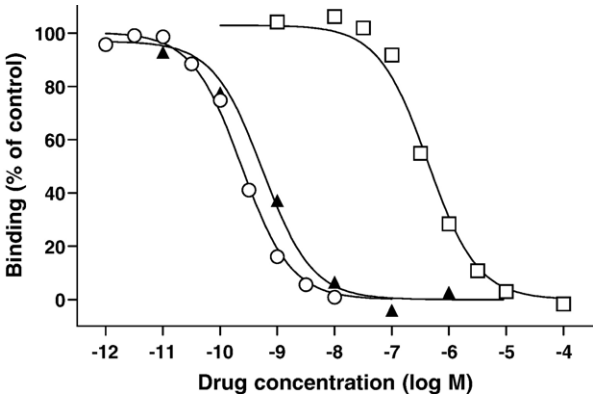

Fig. 3. [^{3}H]vasopressin binding to human mesangial cell plasma membranes is inhibited by nonpeptide vasopressin receptor antagonists. The unlabeled compounds added to the binding assay were: ○, YM218; ▲, SR 49059; □, SR 121463A. Specific binding of [^{3}H]vasopressin is expressed as a percentage of the control binding. Results represent data from three to four independent determinations.

concentration-dependently inhibited by both vasopressin and the vasopressin V_{1A} receptor-selective antagonist, d(CH_2)$_5$Tyr (Me)vasopressin, which exhibited K_i values of 0.74±0.25 nM and 0.48±0.05 nM, respectively (Fig. 2). In contrast, oxytocin and the vasopressin V_2 receptor agonist, dDAVP, showed low affinity for these receptors with K_i values of 9.57±0.78 nM and 23.4±8.4 nM, respectively. YM218 potently inhibited specific binding of [^{3}H]vasopressin to mesangial cell plasma membranes, exhibiting a K_i value of 0.18±0.01 nM (Fig. 3). SR 49059, the vasopressin V_{1A} receptor-selective antagonist, also showed high affinity, exhibiting a K_i value of 0.25±0.03 nM. In contrast, the vasopressin V_2 receptor-selective antagonist, SR 121463A, exhibited much lower affinity, having a K_i value of 308±21 nM. The slopes of the inhibition curves for all compounds did not significantly differ from unity (data not shown).

3.2. YM218 inhibits vasopressin-induced $[Ca^{2+}]_i$ increase in human mesangial cells

Addition of vasopressin to cultured mesangial cells resulted in a rapid increase in $[Ca^{2+}]_i$ that peaked within 10 s and declined gradually to a steady level lasting for at least 5 min.

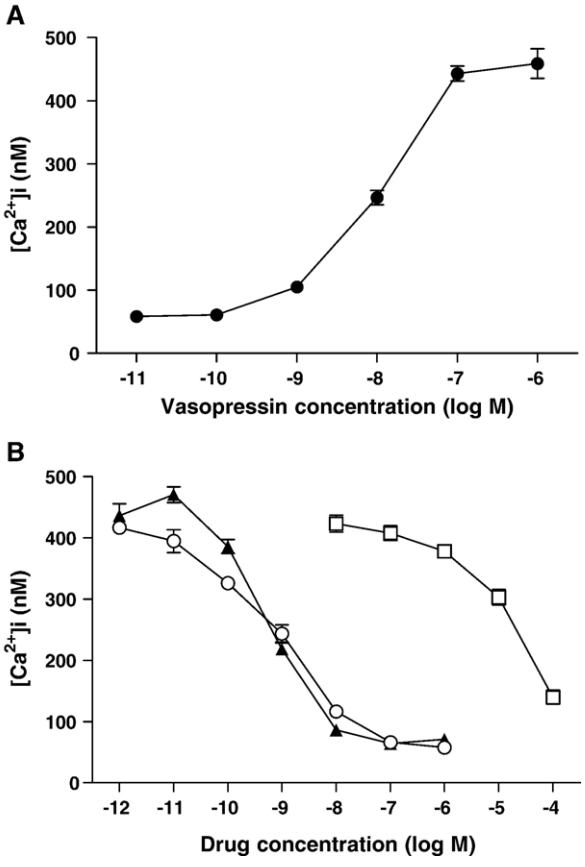

Fig. 4. Nonpeptide vasopressin receptor antagonists inhibit vasopressin-induced $[Ca^{2+}]_i$ increase in human mesangial cells. (A) Effect of vasopressin on $[Ca^{2+}]_i$ in human mesangial cells. (B) Inhibitory effect of vasopressin receptor antagonists on 100 nM vasopressin-induced $[Ca^{2+}]_i$ increase in human mesangial cells. Compounds added to the $[Ca^{2+}]_i$ assay were as follows: ○, YM218; ▲, SR 49059; □, SR 121463A. Values are mean±S.E.M. of four independent determinations.

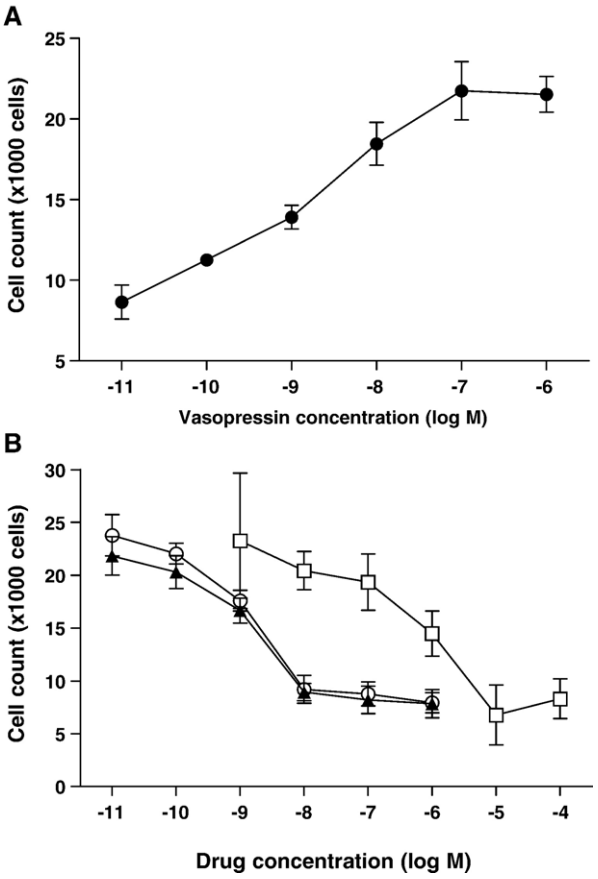

Fig. 5. Effect of YM218 on vasopressin-induced hyperplasia in cultured human mesangial cells. Mesangial cells were incubated for 72 h with (A) increasing concentrations of vasopressin or (B) increasing concentrations of an antagonist and vasopressin (100 nM) in culture medium with 0.5% fetal calf serum and 0.1% bovine serum albumin. Compounds added to the assay were as follows: ○, YM218; ▲, SR 49059; □, SR 121463A. Values are mean±S.E.M. of four independent determinations.

Vasopressin concentration-dependently induced an increase in $[Ca^{2+}]_i$, with an EC_{50} value of 11.2 (8.21–15.1) nM (Fig. 4A). YM218 strongly and concentration-dependently inhibited this $[Ca^{2+}]_i$ increase induced by 100 nM vasopressin, with an IC_{50} value of 0.87 (0.43–1.77) nM (Fig. 4B). When tested under the same experimental conditions, SR 49059 also potently inhibited the vasopressin-induced $[Ca^{2+}]_i$ increase, exhibiting an IC_{50} value of 0.60 (0.44–0.82) nM but SR 121463A did not potently inhibit this response (IC_{50}=2.14 (1.28–3.59) μM). No antagonist elicited any change in basal $[Ca^{2+}]_i$ when tested alone at concentration up to 1 μM (data not shown).

3.3. Effect of YM218 on vasopressin-induced hyperplasia and hypertrophy of human mesangial cells

Vasopressin (10^{-11}–10^{-6} M) caused a concentration-dependent increase in both the cell number and the total protein content of mesangial cells. The EC_{50} values of vasopressin were 1.49 (0.43–5.20) nM for hyperplasia and 3.79 (0.75–19.1) nM for hypertrophy; the maximal stimulation over vehicle-treated control was 2.9-fold for hyperplasia and 1.6-fold for hypertrophy (Figs. 5A, 6A). YM218 concentration-dependently

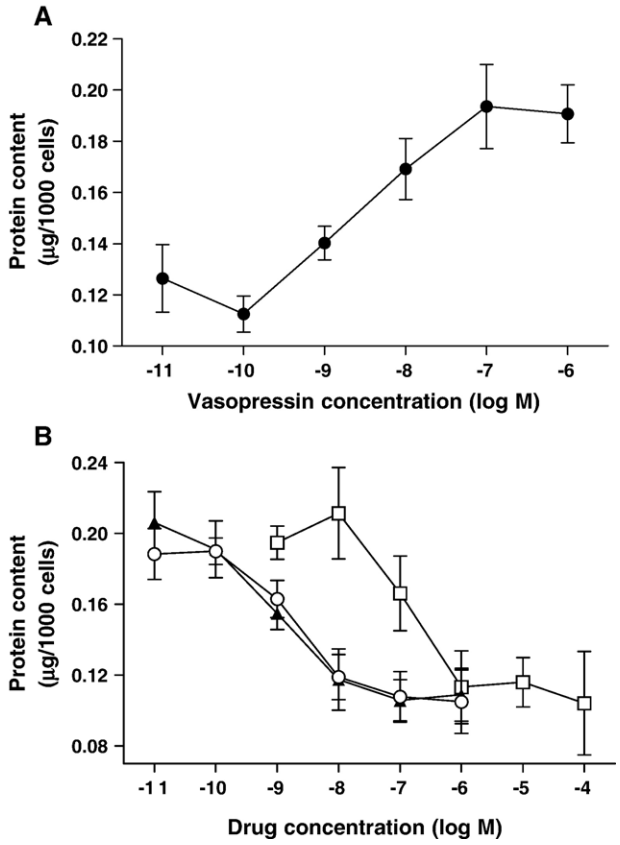

Fig. 6. Effect of YM218 on vasopressin-induced hypertrophy in cultured human mesangial cells. Mesangial cells were incubated for 72 h with (A) increasing concentrations of vasopressin or (B) increasing concentrations of an antagonist and vasopressin (100 nM) in culture medium with 0.5% FC and 0.1% bovine serum albumin. Compounds added to the assay were as follows: ○, YM218; ▲, SR 49059; □, SR 121463A. Values are mean±S.E.M. of four independent determinations.

inhibited the induction of hyperplasia and hypertrophy caused by 100 nM vasopressin, exhibiting IC_{50} values of 2.18 (0.89–5.30) nM against hyperplasia and 1.11 (0.33–3.73) nM against hypertrophy (Figs. 5B, 6B). Furthermore, YM218 itself did not induce hyperplasia or hypertrophy at concentrations up to 1 μM (data not shown). SR 49059 also potently inhibited vasopressin-induced hyperplasia and hypertrophy, exhibiting IC_{50} values of 1.58 (1.15–2.17) nM and 0.83 (0.23–2.95) nM, respectively. In contrast, SR 121463A did not inhibit these effects with much potency (IC_{50} > 100 nM).

4. Discussion

The effects of vasoactive agents on glomerular ultrafiltration are well established (Stockand and Sansom, 1998). One of the pathways involved in the regulation of glomerular perfusion and filtration is thought to be the direct stimulation of contraction in the smooth muscle-like mesangial cells (Schlondorff, 1987). Since glomerular mesangial cells contract after exposure to vasoconstrictive hormones such as vasopressin (Ausiello et al., 1980), this contraction may be responsible for the reduced ultrafiltration coefficients and single-nephron glomerular filtration rates observed after infusion of vasopressin (Ichikawa and Brenner, 1977). Both of these measures of renal function depend on the surface area of the glomerulus; therefore, contraction of mesangial cells in response to vasopressin reduces the glomerular surface area and thus, the filtration rate and the ultrafiltration coefficient (Pfeilschifter, 1989). In addition to this physiologic function, vasopressin induces hyperplasia and hypertrophy, and stimulates the expression of early growth-response genes in, mesangial cells (Ganz et al., 1988; Schulze-Lohoff et al., 1993; Wolthuis et al., 1992). Proliferation and hypertrophy of mesangial cells are hallmarks of many chronic glomerular diseases, thus demonstrating the great need for better understanding of the effects of vasopressin on mesangial cells. Consequently, this study was designed to investigate the possible antimitogenic and antihypertrophic effects of YM218 in cultured human mesangial cells.

Specific binding of [^{3}H]vasopressin to human mesangial cell plasma membranes was specific and saturable. Scatchard plot analysis of equilibrium binding data revealed the existence of a single class of high-affinity binding sites with a K_d of 0.81 nM and a B_{max} of 53.8 fmol/mg protein. The affinity ranking of vasopressin receptor agonists and antagonists indicates a homogenous population of sites exhibiting the expected vasopressin V_{1A} receptor profile comparable to the site previously identified in human vasopressin V_{1A} receptors (Table 1). YM218 potently inhibited [^{3}H]vasopressin binding to human mesangial cell vasopressin V_{1A} receptors; this potency was equal to the potency of SR 49059 and 1700-fold greater than the binding of SR 121463A.

Many of the physiologic responses of mesangial cells to vasopressin, including contraction and cell growth, can be induced by increasing the concentration of intracellular Ca^{2+} and by stimulating protein kinase C (Ganz et al., 1988; Force et al., 1991). In the present experiments, vasopressin induced an increase in $[Ca^{2+}]_i$ concentration-dependently; YM218 potently inhibited this increase. Furthermore, YM218 did not cause an increase in $[Ca^{2+}]_i$ in mesangial cells, indicating a lack of agonistic activity on vasopressin V_{1A} receptors. These results

Table 1
Affinity of vasopressin receptor agonists and antagonists for human mesangial cells and human vasopressin receptors

	K_i (nM)			
	Mesangial cells	Human V_{1A}[a]	Human V_{1B}[a]	Human V_2[a]
AVP	0.74±0.25	0.56±0.11	0.51±0.07	3.27±0.68
Oxytocin	9.57±0.78	5.46±1.97	160±30	1700±340
d(CH_2)$_5$Tyr (Me)AVP	0.48±0.05	0.77±0.18	121±13	113±30
dDAVP	23.4±8.4	62.4±17.6	5.81±1.28	23.3±2.7
YM218	0.18±0.01	0.30±0.02	25500±2500	381±74
SR 49059	0.25±0.03	0.53±0.08	48.4±10.3	178±41
SR 121463A	308±21	304±7	52100±13800	2.75±0.62

Values represent means±S.E.M. obtained from three to five independent experiments performed in duplicate.

[a] Corresponding affinities for human AVP receptors are taken from previously published work (Tahara et al., 1998, 2005).

clearly show that vasopressin is acting through vasopressin V_{1A} receptors, which trigger increases of $[Ca^{2+}]_i$ in human mesangial cells; in contrast, YM218 possesses potent vasopressin V_{1A} receptor antagonistic activity without any measurable agonistic activity.

Proliferation of glomerular mesangial cells and an excessive production of extracellular matrix proteins is associated with proteinuria, glomerulosclerosis and progressive renal failure as tested in numerous animal models (Striker et al., 1989). Additionally, cultured mesangial cells have been shown to respond to vasoactive substances with increased production of various eicosanoids (Williams and Schrier, 1993). Inflammatory mediators, such as interleukin-1 and platelet-derived growth factor, also stimulate eicosanoid production of mesangial cells (Lovett et al., 1983; Scharschmidt and Dunn, 1983). Thus, a variety of agonists regulate the contractile and biosynthetic behavior of mesangial cells. Moreover, cultured mesangial cells exhibit increased proliferation when incubated with cytokines, epidermal growth factor, platelet-derived growth factor, insulin-like growth factor I, transforming growth factor β, interleukins-1, -4 and -6, endothelin-1, angiotensin II, and prostaglandin $F_{2\alpha}$ (Ruef et al., 1990; Mene et al., 1989; Abboud et al., 1987; Lovett et al., 1983). The results of the present study add to this list by clearly showing that vasopressin also induces hyperplasia and hypertrophy, and YM218 potently inhibits vasopressin-induced hyperplasia and hypertrophy in human mesangial cells. Furthermore, although several papers have evidenced the growth inducing effect of vasopressin on rat mesangial cells (Ganz et al., 1988; Wolthuis et al., 1992), this is the first report showing that vasopressin produces concentration-dependent increases in measures of both hyperplasia and hypertrophy in human mesangial cells.

Bardoux et al. (1999) suggest that vasopressin contributes to hyperfiltration, albuminuria and renal hypertrophy in diabetes. Proliferation and hypertrophy of mesangial cells are hallmarks of a variety of renal diseases including diabetic nephropathy. Furthermore, the present results show that vasopressin induces hyperplasia and hypertrophy of human mesangial cells. Therefore, the vasopressin V_{1A} receptor antagonists including YM218 are expected to be effective in the treatment of renal diseases. Indeed, OPC-21268, a vasopressin V_{1A} receptor-selective antagonist, significantly decreased albuminuria without affecting renal function in type 2 diabetic patients (Nishikawa et al., 1996). In contrast, SR 121463, a vasopressin V_2 receptor-selective antagonist, also decreased albuminuria in streptozotocin-induced diabetic rats (Bardoux et al., 2003). At present, the probable involvement of vasopressin in chronic renal diseases including diabetic nephropathy are uncertain and will be subject to further study.

In conclusion, YM218 potently inhibits vasopressin-induced growth responses of human mesangial cells and may have clinical benefit in preventing the development and by inducing regression of mesangial cell growth.

Acknowledgments

The authors thank Drs. Toichi Takenaka, Isao Yanagisawa, Akihiro Tanaka, Hisataka Shikama, Yuichi Iizumi, and Nobuyuki Yamamoto (Astellas Pharma Inc.) for their valuable comments and continuing encouragement.

References

Abboud, H.E., Poptic, E., DiCorieto, P., 1987. Production of platelet-derived growth factor-like protein by rat mesangial cells in culture. J. Clin. Invest. 80, 675–683.

Ahmed, S.A., Gogal Jr., R.M., Walsh, J.E., 1994. A new rapid and simple non-radioactive assay to monitor and determine the proliferation of lymphocytes: an alternative to [^{3}H]thymidine incorporation assay. J. Immunol. Methods 170, 211–224.

Ausiello, D.A., Kreisberg, J.I., Roy, C., Karnovsky, M.J., 1980. Contraction of cultured rat glomerular cells of apparent mesangial origin after stimulation with angiotensin II and arginine vasopressin. J. Clin. Invest. 65, 754–760.

Bakris, G.L., Fairbanks, R., Traish, A.M., 1991. Arginine vasopressin stimulates human mesangial cell production of endothelin. J. Clin. Invest. 87, 1158–1164.

Bardoux, P., Martin, H., Ahloulay, M., Schmitt, F., Bouby, N., Trinh-Trang-Tan, M.M., Bankir, L., 1999. Vasopressin contributes to hyperfiltration, albuminuria, and renal hypertrophy in diabetes mellitus: study in vasopressin-deficient Brattleboro rats. Proc. Natl. Acad. Sci. U. S. A. 96, 10397–10402.

Bardoux, X., Bruneval, P., Heudes, D., Bouby, N., Bnakir, L., 2003. Diabetes-induced albuminuria: role of antidiuretic hormone as revealed by chronic V_2 receptor antagonism in rats. Nephrol. Dial. Transplant. 18, 1755–1763.

Chansel, D., Czekalski, S., Pham, P., Ardaillou, R., 1992. Characterization of angiotensin II receptor subtypes in human glomeruli and mesangial cells. Am. J. Physiol. 262, F432–F441.

Force, T., Kyriakis, J.M., Avruch, J., Bonventre, J.V., 1991. Endothelin, vasopressin, and angiotensin II enhance tyrosine phosphorylation by protein kinase C-dependent and -independent pathways in glomerular mesangial cells. J. Biol. Chem. 266, 6650–6656.

Ganz, M.B., Pekar, S.K., Perfetto, M.C., Sterzel, R.B., 1988. Arginine vasopressin promotes growth of rat glomerular mesangial cells in culture. Am. J. Physiol. 255, F898–F906.

Ichikawa, I., Brenner, B.M., 1977. Evidence for glomerular actions of ADH and dibutyryl cyclic AMP in the rat. Am. J. Physiol. 233, F103–F113.

Kashgarian, M., Sterzel, R.B., 1992. The pathobiology of the mesangium. Kidney Int. 41, 524–529.

Kurihara, I., Saito, T., Obara, K., Shoji, Y., Hirai, M., Soma, J., Sato, H., Imai, Y., Abe, K., 1996. Effect of a nonpeptide vasopressin V1 antagonist (OPC-21268) on experimental accelerated focal glomerulosclerosis. Nephron 73, 629–636.

Lovett, D.H., Ryan, J.L., Sterzel, R.B., 1983. Stimulation of rat mesangial cell proliferation by macrophage interleukin 1. J. Immunol. 131, 2830–2836.

Mene, P., Simonson, M.S., Dunn, M.J., 1989. Physiology of the mesangial cell. Physiol. Rev. 69, 1347–1424.

Nishikawa, T., Omura, M., Iizuka, T., Saito, I., Yoshida, S., 1996. Short-term clinical trial of 1-(1-[4-(3-acetylaminopropoxy)-benzoyl]-4-piperidyl)-3,4-dihydro-2(1H)-quinolinone in patients with diabetic nephropathy. Possible effectiveness of the specific vasopressin V1 receptor antagonist for reducing albuminuria in patients with non-insulin dependent diabetes mellitus. Arzneim.-Forsch. 46, 875–878.

Orth, S.R., Weinreich, T., Bonisch, S., Weih, M., Ritz, E., 1995. Angiotensin II induces hypertrophy and hyperplasia in adult human mesangial cells. Exp. Nephrol. 3, 23–33.

Otsuka, F., Ogura, T., Yamauchi, T., Oishi, T., Hashimoto, M., Mimura, Y., Makino, H., 1997. Effects of OPC-21268, a vasopressin V1-receptor antagonist, on expression of growth factors from glomeruli in spontaneously hypertensive rats. Regul. Pept. 72, 87–95.

Pfeilschifter, J., 1989. Cross-talk between transmembrane signaling systems: a prerequisite for the delicate regulation of glomerular haemodynamics by mesangial cells. Eur. J. Clin. Investig. 19, 347–361.

Rebibou, J.M., He, C.J., Delarue, F., Peraldi, M.N., Adida, C., Rondeau, E., Sraer, J.D., 1992. Functional endothelin 1 receptors on human glomerular podocytes and mesangial cells. Nephrol. Dial. Transplant. 7, 288–292.

Ruef, C., Budde, K., Lacy, J., Northemann, W., Baumann, M., Sterzel, R.B., Coleman, D.L., 1990. Interleukin 6 is an autocrine growth factor for mesangial cells. Kidney Int. 38, 249–257.

Scharschmidt, L.A., Dunn, M.J., 1983. Prostaglandin synthesis by rat glomerular mesangial cells in culture. J. Clin. Invest. 71, 1756–1764.

Schlondorff, D., 1987. The glomerular mesangial cell: an expanding role for a specialized pericyte. FASEB J. 1, 272–281.

Schulze-Lohoff, E., Kohler, M., Fees, H., Reindl, N., Sterzel, R.B., 1993. Divergent effects of arginine vasopressin and angiotensin II on proliferation and expression of the immediate early genes c-fos, c-jun and Egr-1 in cultured rat glomerular mesangial cells. J. Hypertens. 11, 127–134.

Serradeil-Le Gal, C., Wagnon, J., Garcia, C., Lacour, C., Guiraudou, P., Christophe, B., Villanova, G., Nisato, D., Maffrand, J.P., Le Fur, G., Guillon, G., Cantau, B., Barberis, C., Trueba, M., Ala, Y., Jard, S., 1993. Biochemical and pharmacological properties of SR 49059, a new, potent, nonpeptide antagonist of rat and human vasopressin V_{1a} receptors. J. Clin. Invest. 92, 224–231.

Serradeil-Le Gal, C., Lacour, C., Valette, G., Garcia, G., Foulon, L., Galindo, G., Bankir, L., Pouzet, B., Guillon, G., Barberis, C., Chicot, D., Jard, S., Vilain, P., Garcia, C., Marty, E., Raufaste, D., Brossard, G., Nisato, D., Maffrand, J.P., Le Fur, G., 1996. Characterization of SR 121463A, a highly potent and selective, orally active vasopressin V_2 receptor antagonist. J. Clin. Invest. 98, 2729–2738.

Serradeil-Le Gal, C., Wagnon, J., Simiand, J., Griebel, G., Lacour, C., Guillon, G., Barberis, C., Brossard, G., Soubrie, P., Nisato, D., Pascal, M., Pruss, R., Scatton, B., Maffrand, J.P., Le Fur, G., 2002. Characterization of (2S,4R)-1-[5-Chloro-[(2,4-dimethoxyphenyl)-sulfonyl]-3-(2-methoxy-phenyl)-2-oxo-2,3-dihydro-1H-indol-3-yl]-4-hydroxy-*N*,*N*-dimethyl-2-pyrrolidine carboxamide (SSR149415), a selective and orally active vasopressin V_{1b} receptor antagonist. J. Pharmacol. Exp. Ther. 300, 1122–1130.

Simonson, M.S., Osanai, T., Dunn, M.J., 1991. The effects of endothelin on cytosolic calcium in cultured human and rat glomerular mesangial cells. Clin. Invest. Med. 14, 499–507.

Stockand, J.D., Sansom, S.C., 1998. Glomerular mesangial cells: electrophysiology and regulation of contraction. Physiol. Rev. 78, 723–744.

Striker, L.J., Doi, T., Elliot, S., Striker, G.E., 1989. The contribution of glomerular mesangial cells to progressive glomerulosclerosis. Semin. Nephrol. 9, 318–328.

Tahara, A., Tomura, Y., Wada, K., Kusayama, T., Tsukada, J., Takanashi, M., Yatsu, T., Uchida, W., Tanaka, A., 1997. Pharmacological profile of YM087, a novel potent nonpeptide vasopressin V_{1A} and V_2 receptor antagonist, in vitro and in vivo. J. Pharmacol. Exp. Ther. 282, 301–308.

Tahara, A., Saito, M., Sugimoto, T., Tomura, Y., Wada, K., Kusayama, T., Tsukada, J., Ishii, N., Yatsu, T., Uchida, W., Tanaka, A., 1998. Pharmacological characterization of the human vasopressin receptor subtypes stably expressed in Chinese hamster ovary cells. Br. J. Pharmacol. 125, 1463–1470.

Tahara, A., Tsukada, J., Tomura, Y., Kusayama, T., Wada, K., Ishii, N., Taniguchi, N., Suzuki, T., Yatsu, T., Uchida, W., Shibasaki, M., 2005. Effects of YM218, a nonpeptide vasopressin V_{1A} receptor-selective antagonist, on human vasopressin and oxytocin receptors. Pharmacol. Res. 51, 275–281.

Thibonnier, M., Bayer, A.L., Leng, Z., 1993. Cytoplasmic and nuclear signaling pathways of V1-vascular vasopressin receptors. Regul. Pept. 45, 79–84.

Tsukada, J., Tahara, A., Tomura, Y., Wada, K., Kusayama, T., Ishii, N., Aoki, M., Yatsu, T., Uchida, W., Taniguchi, N., Tanaka, A., 2002. Pharmacological characterization of YM471, a novel potent vasopressin V_{1A} and V_2 receptor antagonist. Eur. J. Pharmacol. 446, 129–138.

Williams, B., Schrier, R.W., 1993. Glucose-induced protein kinase C activity regulates arachidonic acid release and eicosanoid production by cultured glomerular mesangial cells. J. Clin. Invest. 92, 2889–2896.

Wolthuis, A., Boes, A., Rodemann, H.P., Grond, J., 1992. Vasoactive agents affect growth and protein synthesis of cultured rat mesangial cells. Kidney Int. 41, 124–131.