Inhibition of Vasopressin Action in Vascular Smooth Muscle by the V1 Antagonist OPC-21268

Xiaomei Li, Andreas Kribben, Eric D. Wieder, Phoebe Tsai, Raphael A. Nemenoff, Robert W. Schrier

Abstract In vascular smooth muscle cells arginine vasopressin acting through the V1 receptor increases intracellular Ca2+, leading to vasoconstriction. Recent studies have also shown that vasopressin activates mitogen-activated protein kinase (MAP kinase), which may contribute to vasopressin-induced hypertrophy of vascular smooth muscle cells. We examined the ability of an orally active, nonpeptide selective V1 antagonist (OPC-21268) to block vasopressin binding and postreceptor signaling in these cells. [3H]Vasopressin binding at 2×10⁻⁹ mol/L was half-maximally blocked at 10⁻⁴ mol/L OPC-21268. To compare effects of OPC-21268 on binding and postreceptor signaling, we stimulated cells with 10⁻⁴ mol/L vasopressin. At this vasopressin concentration, half-maximal inhibition of binding occurred at 5×10⁻⁵ mol/L OPC-21268. Half-maximal inhibition of Ca²⁺ efflux or increases in intracellular free Ca²⁺ required higher concentrations of antagonist (10⁻⁷ mol/L), and half-maximal inhibition of vasopressin-stimulated MAP kinase was observed only at 10⁻⁶ mol/L OPC-21268. These results indicate that this agent selectively blocks both vasopressin binding and postreceptor signaling in vascular smooth muscle cells. The requirement of higher concentrations of OPC-21268 for blocking increases in intracellular Ca²⁺ and activation of MAP kinase suggests that binding to a fraction of V1 receptors generates maximal levels of second messengers or the existence of subtypes of the V1 receptor with differential affinity for this antagonist. These data have implications for the clinical use of this compound. (Hypertension. 1994;23:117-222.)

Key Words • argipressin • calcium • protein kinases • muscle, smooth, vascular

Received May 6, 1993; accepted in revised form October 26, 1993.
From the Department of Medicine, University of Colorado School of Medicine, Denver.
Reprint requests to Raphael A. Nemenoff, PhD, Department of Medicine, University of Colorado Health Sciences Center, Box C281, 4200 E 9th Ave, Denver, CO 80262.

arginine vasopressin (AVP) has been shown to play an important physiological role in vasoconstriction and antidiuresis. Classically, two classes of AVP receptors have been identified.¹ The V1 receptor, which has been described in vascular smooth muscle and liver, signals through increases in intracellular Ca2⁺; the V2 receptor, which has been described in the kidney, signals through increases in cyclic AMP. Both of these receptors have been cloned recently and belong to the family of “seven membrane spanning” receptors, which signal through G proteins. In vascular smooth muscle cells (VSMCs), acute stimulation by AVP results in a rapid contraction of the cells.² Prolonged AVP stimulation of VSMCs in the absence of other growth-promoting agents has been shown to cause hypertrophy.³ Although a number of postreceptor signaling events regulated by AVP have been described, the mechanisms of AVP-induced contraction and hypertrophy have not been clearly delineated.

In VSMCs, AVP acting through a pertussis-insensitive G protein causes activation of phosphatidylinositol-specific phospholipase C-β, which mobilizes intracellular Ca²⁺ and through the production of diacylglycerol activates protein kinase C. Increases in intracellular Ca²⁺, through the action of calmodulin, activate myosin light chain kinase, which in turn phosphorylates myosin light chain, a critical event in the early phase of contraction. Recently it has been shown that AVP and other vasoconstrictors induce the activation of p42/44 mitogen-activated protein kinase (MAP kinase).⁴ p42/44 MAP kinases are members of a family of serine-threonine protein kinases that are themselves activated by phosphorylation on tyrosine and threonine residues. These kinases are activated by growth factors and are believed to play a critical role in cell proliferation (for a recent review, see Reference 15).

A number of specific AVP analogues have been described.⁵ These molecules are all peptides, and thus their clinical efficacy is likely to be limited to parenteral administration. Recently, Yamamura et al⁶ identified an orally active, nonpeptide V1 antagonist, OPC-21268. This agent selectively inhibited AVP binding in rat liver plasma membranes. In the present study we have examined the potency of this agent to inhibit AVP binding and postreceptor signaling events in cultures of rat aortic VSMCs.

Methods

The V1 antagonist OPC-21268 (1-[1-(3-acetylamino-propoxy)benzoyl]-4-piperidyl)-3,4-dihydro-2(1H)-quinolinone) and V2 antagonist OPC-31260 ((±)-5-dimethylamino-1-[4-(2-methylbenzoylamino)benzoyl]-2,3,4,5-tetrahydro-1H-benzazepine hydrochloride) were obtained from Otsuka Pharmaceutical Co (Japan). Collagenase was obtained from Boehringer Mannheim (Germany); Eagle’s minimum essential medium (MEM) was from Gibco (Grand Island, NY); and HEPES, AVP, phorbol 12-myristate 13-acetate, myelin basic protein (MBP), protein kinase inhibitor peptide, phenylmethylsulfonyl fluoride (PMSF), and orthovanadate were obtained from Sigma Chemical Co (St Louis, Mo). [3H]AVP was obtained from New England Nuclear (Boston, Mass); [γ-³²P]ATP was from Amersham (Arlington Heights, Ill). ⁴Ca was from ICN Biomedicals Inc (Irvine, Calif). Fura 2-AM and pluronic F127 were obtained from Molecular
Probes Inc (Eugene, Ore). Bradford protein assay kit was obtained from Bio-Rad (Richmond, Calif).

**Vascular Smooth Muscle Cell Culture**

Rat aortic VSMCs were isolated and cultured as previously described in detail. Briefly, under sterile conditions, aortas were dissected from male Sprague-Dawley rats (200 to 300 g) and cleaned of adventitia and connective tissue. The vessels were chopped and incubated for 2 hours in Eagle’s MEM containing 2.4 mg/ml collagenase. The resulting cell suspension was plated on 35-mm dishes and grown in Eagle’s MEM containing 2 mmol/L L-glutamine, 2 g/L NaHCO3, 100 IU/mL penicillin, 100 mg/L streptomycin, and 10% fetal calf serum at 37°C in a humidified atmosphere of 95% air and 5% CO2. Cells were passaged using trypsin-EDTA. Cells (passages 1 through 9) were grown to confluence and serum-restricted 20 hours before study. For studies measuring intracellular Ca2+, cells were grown on glass cover slips.

**Receptor Binding**

AVP-specific binding was determined as previously described. Cells were washed twice with ice-cold binding buffer (mmol/L: NaCl, 120; KCl, 3; MgSO4, 1.2; CaCl2, 1; KH2PO4, 1.25; glucose, 10; HEPES, 25; and 0.1% bovine serum albumin, pH 7.4). Cells were incubated for 90 minutes at 4°C in 1 mL binding buffer containing 2×10-9 mol/L [3H]AVP (except where indicated) in the presence or absence of different concentrations (10-6 to 10-1 mol/L) of OPC-21268 or nonradioactive AVP. Reactions were terminated by aspirating the solution, and the cells were rapidly rinsed four times with 2 mL cold washing buffer to remove unbound [3H]AVP. Cells were solubilized with 0.1% sodium dodecyl sulfate in 0.1N NaOH, and radioactivity was measured in a liquid scintillation counter (Tri-Carb 460C, Packard Instrument Co Inc, Downers Grove, Ill). Specific binding was determined by subtraction of nonspecific binding—[3H]AVP bound in the presence of 10-5 mol/L AVP.

**Calcium Efflux**

Ca2+ efflux was determined by a method similar to that previously described. The culture medium was aspirated and cells were washed twice with 2 mL physiological salt solution (PSS) (mmol/L: NaCl, 140; KCl, 4.6; MgCl2, 1.0; CaCl2, 2.8; glucose, 10; HEPES, 10, pH 7.4). Cells were loaded with 5 mol/L Ca2+ in 1 mL PSS for 2 hours at 37°C. At the end of the incubation, cells were rinsed 10 times rapidly with 1 mL PSS and 1 mL fresh PSS added. The PSS was removed at 1-minute intervals and replaced with fresh buffer for 4 minutes. At 4 and 5 minutes, buffer was replaced with PSS containing AVP or vehicle and OPC-21268. After the addition of AVP, buffer was removed at 2-minute intervals and replaced with fresh PSS. At 14 minutes, the cell pellets were solubilized with 0.1% sodium dodecyl sulfate in 0.1N NaOH. Buffer samples and solubilized cells were collected in vials for liquid scintillation counting. Ca2+ release from the cells was expressed as a percentage of total available cellular radioactivity (counts released after AVP addition plus counts in solubilized cell pellet).

**Quantitation of Intracellular Free Ca2+ Concentration**

[Ca2+]i in single VSMCs was measured with the Ca2+ indicator fura 2 using video imaging. VSMCs were grown on No. 1 glass coverslips and loaded with 5 mmol/L fura 2-AM in the presence of the nonionic detergent pluronic (0.025%) at 37°C for 30 minutes. The VSMCs were then washed to remove extracellular fura 2-AM. OPC-21268 was added 2 minutes before the addition of AVP (10-4 mol/L). Fluorescence in individual cells was measured and analyzed with a customized imaging system (S&M Microscopes, Colorado Springs, Colo.). The coverslip was mounted in an open chamber on the stage of an inverted microscope (Diaphot, Nikon, Garden City, NY) equipped for epifluorescence microscopy. The VSMCs were illuminated by a 75-W xenon lamp alternately with a 340- and 380-nm excitation filter (340DF20 and 380DF10, Omega Optical Inc, Brattleboro, Vt) and a 400-nm dichroic mirror (DM 400, Nikon). These excitation filters were changed using a computer-controlled filter wheel (1meic, Charlestown, Mass). Emitted light was collected through the dichroic mirror, transmitted through a 510-nm filter (510 DF20, Omega Optical), and imaged onto the face of an intensified charge-coupled device camera (Genisys Intensifier, CCD72 camera, Dage-MTI, Inc, Michigan City, Ind). The images were processed through an IBM PC/AT computer using an image-processing program (Image1 Fluor, Universal Imaging Corp, West Chester, Pa). Autofluorescence measured in unloaded cells was less than 2% of the fura 2 signal. The maximal ratio was measured after addition of 10 nmol/L ionomycin and the minimal ratio after subsequent addition of 10 nmol/L Triton X-100. [Ca2+]i was calculated using the formula of Grynkiewicz, with an estimated Kd:β for fura 2 of 1.8 nmol/L.

**Mitogen-Activated Protein Kinase Activity**

Cells were pretreated for 2 minutes in Eagle’s MEM with various concentrations of OPC-21268 and then stimulated with 10-8 mol/L AVP for 5 minutes at 37°C. Stimulation was stopped by aspirating the media. Cells were rinsed twice with ice-cold phosphate-buffered saline and lysed by the addition of 0.25 mL lysis buffer (mmol/L: β-glycerophosphate, 50; Na3VO4, 0.1; MgCl2, 2.0; EGTA, 1.0; dithiothreitol, 1.0; pepstatin, 0.02; leupeptin, 0.02; PMSF, 1; as well as 0.5% [wt/vol] Trition X-100 and 100 mM α-mannosidase, pH 7.2). Lysates were centrifuged for 10 minutes at 12,000g. Supernatants were assayed for MAP kinase activity by phosphorylation of MBP using a modification of the method previously described. The assay was performed at 30°C for 15 minutes in a final volume of 40 μL containing (final concentration) 12.5 mmol/L MgCl2, 50 μg/mL protein kinase inhibitor peptide, 1 mg/mL MBP, and 50 mmol/L [γ-32P]ATP. Reactions were terminated by the addition of 10 μL of 25% (wt/vol) trichloroacetic acid. Aliquots (25 μL) of the acidified reaction mixtures were spotted onto 1.5-cm2 phosphocellulose paper (Whatman P-81). After four washes in 75 mmol/L phosphoric acid and one wash in acetonitrile, 32P-labeled protein was counted by liquid scintillation. MAP kinase activity was expressed as picomoles of 32P per minute per milligram of protein.

**Statistical Analysis**

Results are expressed as mean ± SEM. Differences between means were evaluated by ANOVA with the Newman-Keuls multiple-comparisons procedure. A value of P<.05 was taken to be significant.

**Results**

**Vasopressin Binding**

The ability of the V1 antagonist OPC-21268 to inhibit [3H]AVP-specific binding is shown in Fig. 1. At 2×10-9 mol/L [3H]AVP, approximately 25% of the total counts were bound, with nonspecific binding (binding in the presence of 10-4 mol/L nonradioactive AVP) representing 5% of the total counts. Half-maximal inhibition of specific binding was obtained at 10-9 mol/L OPC-21268, and specific binding was completely inhibited at 10-6 mol/L antagonist. The potency of this agent to inhibit binding in these cells is comparable to that of the peptide V1 antagonist developed by Manning et al. OPC-21268 was more potent than nonradioactive AVP in displacing [3H]AVP, with half-maximal displacement occurring at approximately 10-7 mol/L AVP. The dose-
Inhibition of AVP Action in VSMC

Li et al

Inhibition of AVP Action

Specific Binding ( % )

-log Concentration (M)

FIG 1. Plot shows inhibition of [3H]arginine vasopressin (3H-AVP) binding by OPC-21268 (OPC) and AVP. Cells were incubated with 2x10^-6 mol/L [3H]AVP in the absence or presence of indicated concentration of OPC-21268 or nonradioactive AVP for 90 minutes at 4°C. Nonspecific binding was determined by [3H]AVP binding in the presence of 10^-6 mol/L nonradioactive AVP. Specific binding was calculated as the difference between total and nonspecific binding. Binding in the absence of OPC-21268 was defined as 100%. Data represent the mean of triplicate determinations of three experiments with SEM indicated.

response curve for displacement of [3H]AVP by OPC-21268 appeared to be broader than that for AVP. To determine if this was due to multiple binding sites for OPC-21268, we analyzed the binding data using GRAPH-PAD software for a one-site or two-site fit. The fit obtained with a two-site model was not significantly better than a one-site model (P = 0.1627, data not shown). The V2 antagonist OPC-31260 at a concentration of 10^-6 mol/L did not significantly inhibit [3H]AVP binding in these cells, indicating that the V2 receptor is the major form in VSMCs (data not shown). To compare the ability of OPC-21268 to block binding and postreceptor signaling (see below), we repeated the binding inhibition at 10^-8 mol/L [3H]AVP, the concentration used for measuring changes in Ca^{2+} and MAP kinase (see Fig 5).

\[ ^{4}\text{Ca}^{2+} \text{ Efflux} \]

Measurements of \(^{4}\text{Ca}^{2+}\) efflux have been used as an index of increases in intracellular Ca^{2+}. Fig 2 shows the ability of OPC-21268 to block AVP-induced Ca^{2+} efflux in VSMCs. Stimulation of cells with 10^-8 mol/L AVP resulted in a threefold increase in \(^{4}\text{Ca}^{2+}\) efflux. Half-maximal inhibition of this effect was obtained between 10^-8 and 10^-7 mol/L OPC-21268, and Ca^{2+} efflux was completely blocked at 10^-6 mol/L antagonist. Stimulation of Ca^{2+} efflux by angiotensin II was not inhibited even at 10^-5 mol/L OPC-21268, indicating that the drug does not affect postreceptor events mediating the increase in intracellular Ca^{2+}. The V1 antagonist OPC-31260 was ineffective in blocking Ca^{2+} efflux (data not shown).

Intracellular Ca^{2+}

The ability of OPC-21268 to block AVP-induced increases in [Ca^{2+}], was determined by measuring fluorescence changes with fura 2 in single cells using a video-imaging technique. Studies were performed on fields of subconfluent cells grown on glass coverslips, with 10 to 20 cells in a field. AVP (10^-8 mol/L) induced a rapid increase in [Ca^{2+}], within 20 seconds (Fig 3). Approximately 80% of the cells showed greater than a 0.2 change in fluorescence ratio. Levels of [Ca^{2+}], remained elevated for at least 2 minutes and returned to basal values by 5 minutes. Pretreatment of VSMCs with OPC-21268 decreased the maximal [Ca^{2+}], achieved by AVP. Half-maximal inhibition of the rise in [Ca^{2+}], was obtained between 10^-8 and 10^-7 mol/L OPC-21268, and complete inhibition occurred at 10^-6 mol/L antagonist. These higher OPC-21268 concentrations (10^-7 or 10^-6 mol/L) also appeared to increase the time required after AVP addition to reach maximal [Ca^{2+}], (Fig 3).

Mitogen-Activated Protein Kinase

We next examined the ability of the V1 antagonist to inhibit AVP-induced activation of MAP kinase. After stimulation of cells with 10^-8 mol/L AVP, cell lysates were prepared and assayed for MAP kinase using the specific substrate MBP, as previously described.13,14 As

\[ ^{4}\text{Ca}^{2+} \text{ Efflux} \]

Measurements of \(^{4}\text{Ca}^{2+}\) efflux have been used as an index of increases in intracellular Ca^{2+}. Fig 2 shows the ability of OPC-21268 to block AVP-induced Ca^{2+} efflux in VSMCs. Stimulation of cells with 10^-8 mol/L AVP resulted in a threefold increase in \(^{4}\text{Ca}^{2+}\) efflux. Half-maximal inhibition of this effect was obtained between 10^-8 and 10^-7 mol/L OPC-21268, and Ca^{2+} efflux was completely blocked at 10^-6 mol/L antagonist. Stimulation of Ca^{2+} efflux by angiotensin II was not inhibited even at 10^-5 mol/L OPC-21268, indicating that the drug does not affect postreceptor events mediating the increase in intracellular Ca^{2+}. The V1 antagonist OPC-31260 was ineffective in blocking Ca^{2+} efflux (data not shown).

Intracellular Ca^{2+}

The ability of OPC-21268 to block AVP-induced increases in [Ca^{2+}], was determined by measuring fluorescence changes with fura 2 in single cells using a video-imaging technique. Studies were performed on fields of subconfluent cells grown on glass coverslips, with 10 to 20 cells in a field. AVP (10^-8 mol/L) induced a rapid increase in [Ca^{2+}], within 20 seconds (Fig 3). Approximately 80% of the cells showed greater than a 0.2 change in fluorescence ratio. Levels of [Ca^{2+}], remained elevated for at least 2 minutes and returned to basal values by 5 minutes. Pretreatment of VSMCs with OPC-21268 decreased the maximal [Ca^{2+}], achieved by AVP. Half-maximal inhibition of the rise in [Ca^{2+}], was obtained between 10^-8 and 10^-7 mol/L OPC-21268, and complete inhibition occurred at 10^-6 mol/L antagonist. These higher OPC-21268 concentrations (10^-7 or 10^-6 mol/L) also appeared to increase the time required after AVP addition to reach maximal [Ca^{2+}], (Fig 3).

Mitogen-Activated Protein Kinase

We next examined the ability of the V1 antagonist to inhibit AVP-induced activation of MAP kinase. After stimulation of cells with 10^-8 mol/L AVP, cell lysates were prepared and assayed for MAP kinase using the specific substrate MBP, as previously described.13,14 As
shown in Fig 4, AVP induced a fourfold increase in MAP kinase activity. Half-maximal inhibition of MAP kinase activation was observed at approximately 10⁻⁶ mol/L OPC-21268 and complete inhibition at 10⁻⁵ mol/L. Epidermal growth factor stimulation of MAP kinase was not affected by 10⁻⁵ mol/L antagonist. The V₂ antagonist OPC-31260 was ineffective in blocking AVP-induced MAP kinase (data not shown).

Fig 5 summarizes the dose-response curves for inhibition of AVP-binding, Ca²⁺ efflux, increases in [Ca²⁺], and activation of MAP kinase by OPC-21268, all measured after stimulation by 10⁻⁸ mol/L AVP. This AVP concentration was chosen to allow the comparison of the potency of OPC-21268 in blocking both binding and postreceptor signaling pathways. The dose-response curves for both Ca²⁺ efflux elevations and increases in [Ca²⁺], determined by fura 2 are superimposable, with half-maximal inhibition at 10⁻⁷ mol/L OPC-21268.

Discussion

The V₁ isoform of the AVP receptor is believed to be the major form present in vascular smooth muscle and to mediate the effects of AVP on cell contraction and hypertrophy. The receptor subtypes in these cells have not been clearly delineated. In the present studies we have shown that a nonpeptide specific V₁ antagonist, OPC-21268, blocks both AVP binding and postreceptor signaling events in cultured aortic VSMCs. A specific V₂ antagonist was ineffective in blocking these effects. The potency of OPC-21268 in blocking specific [³H]AVP binding was similar to that of specific peptide V₁ antagonists used in earlier studies. Binding inhibition occurred at much lower concentrations than observed in binding to liver membranes.

OPC-21268 also specifically blocked two postreceptor signaling events stimulated by AVP: increases in intracellular Ca²⁺ and activation of MAP kinase. AVP-induced increases in intracellular Ca²⁺ were measured using two techniques: ⁴⁵Ca²⁺ efflux and increases in [Ca²⁺], using fura 2. Both techniques gave the same results, requiring 20 to 50 times higher concentrations of antagonist to half-maximally block Ca²⁺ increases than to half-maximally inhibit binding. These data suggest the existence of "spare" receptors. In this model maximal increases in intracellular Ca²⁺ occur when only a small number of AVP receptors are occupied. High concentrations of antagonist thus are required to block AVP binding to most of the receptors before inhibition of postreceptor events is detected. Previous studies have examined the AVP dose response for increasing intracellular Ca²⁺ in VSMCs. Half-maximal stimulation was obtained at 5×10⁻⁷ to 5×10⁻⁸ mol/L AVP. These are similar to or higher than AVP concentrations required for half-maximal AVP binding, implicating a
threshold phenomenon, in which occupancy of less than a certain number of receptors does not result in any Ca\(^{2+}\) increase. Reconciling these two models will require additional studies correlating the production of inositol 1,4,5-trisphosphate with changes in intracellular Ca\(^{2+}\). Matsui and coworkers also have recently examined the ability of OPC-21268 to block AVP-induced increases in intracellular Ca\(^{2+}\). In their study half-maximal increases in intracellular Ca\(^{2+}\) were obtained between 10\(^{-8}\) and 10\(^{-7}\) mol/L AVP; 10\(^{-6}\) mol/L OPC-21268 completely blocked Ca\(^{2+}\) increases in the presence of 10\(^{-5}\) mol/L AVP. The dose-response curve for OPC-21268 was not examined in their study.

Inhibition of AVP-induced activation of MAP kinase required much higher concentrations of OPC-21268 than either inhibition of Ca\(^{2+}\) increases or AVP binding. Maximal activation of MAP kinase can be achieved under conditions in which AVP-induced Ca\(^{2+}\) increases are inhibited by greater than 70% (Fig 5). These data suggest that increases in intracellular Ca\(^{2+}\) are not required for MAP kinase activation and are consistent with earlier studies showing that activation of protein kinase C was both necessary and sufficient for MAP kinase activation. These data are also consistent with the concept of "spare" receptors for MAP kinase activation. The AVP dose-response curve for MAP kinase activation is shifted to the left compared with AVP binding, with half-maximal activation occurring at approximately 10\(^{-10}\) mol/L AVP. Therefore, maximal MAP kinase activation is achieved at low receptor occupancy, presumably through sufficient diglyceride production to maximally activate protein kinase C.

An alternative model to "spare" receptors that could account for the differential effects of OPC-21268 is the existence of multiple subtypes of V\(_1\) receptors with similar affinities for AVP but different affinities for OPC-21268. One subtype would be coupled to increases in intracellular Ca\(^{2+}\) and activation of MAP kinase and would have a low affinity for OPC-21268. The second subtype with higher affinity for the antagonist would be coupled to as yet unidentified second messenger systems or act as a clearance receptor for AVP in vascular smooth muscle. Analysis of the binding displacement (Fig 1) by a curve-fitting program did not allow us to distinguish between a one-site or two-site model. Further testing of multiple binding sites for OPC-21268 will require binding studies using radiolabeled antagonist. It should be noted that polymeric chain reactions using RNA from rat liver and oligonucleotides derived from the published sequence of the V\(_1\) receptor yield multiple bands (L.E. Heasley, personal communication), suggesting the existence of multiple isoforms of the V\(_1\) receptor. OPC-21268 may prove to be a valuable agent in identifying these novel subtypes and establishing their physiological role. It is also possible that pretreatment of VSMCs with OPC-21268 results in internalization of a portion of the AVP receptors, with higher concentrations of antagonists required to inhibit the receptors remaining at the cell surface. Examination of this will require binding studies using radiolabeled OPC-21268.

The existence of receptor subtypes with differential affinity for nonpeptide antagonists has been demonstrated for angiotensin II: DuP 753 shows selectivity for the AT\(_1\) receptor and PD 123177 for the AT\(_2\) receptor (see Reference 26 for review). Interestingly, both subtypes have been shown to exist in the same tissue, and the physiological role of the AT\(_2\) receptor remains to be established.

In summary, we have shown that OPC-21268 selectively inhibits AVP binding and postreceptor signaling in VSMCs with different potencies. The ability of certain concentrations of OPC-21268 to selectively block certain postreceptor events will be a powerful tool in dissecting the specific events required to mediate AVP-induced contraction versus hypertrophy in these cells. These data also suggest that the dose of this drug used clinically will be a critical determinant in obtaining therapeutic effects.

Acknowledgments

This work was supported by grants from Otsuka Pharmaceutical Co, the National Institutes of Health (DK 19928, DK 39902), and the Markey Foundation. X.-M. Li was supported by a Fellowship from the International Society of Nephrology. We thank Carolyn Burke for assistance with the figures.

References


Inhibition of vasopressin action in vascular smooth muscle by the V1 antagonist OPC-21268.
X Li, A Kribben, E D Wieder, P Tsai, R A Nemenoff and R W Schrier

Hypertension. 1994;23:217-222
doi: 10.1161/01.HYP.23.2.217

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1994 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/23/2/217

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at: http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at: http://hyper.ahajournals.org/subscriptions/