Effects of a Nonpeptide Vasopressin Antagonist (OPC-21268) on Cytosolic Ca\textsuperscript{2+} Concentration in Vascular and Cardiac Myocytes

Hiroshi Matsui, Osami Kohmoto, Yasunobu Hirata, and Takashi Serizawa

A selective V\textsubscript{1} antagonist, l-{[4(3-acetylamino)propoxy]benzoyl}-4-piperidyl)-3,4-dihydro-2(1H)-quinolinone (OPC-21268), which is nonpeptide and orally effective, has been recently synthesized. We studied the effects of vasopressin and OPC-21268 on cell contraction with a video motion detector and cytosolic Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]\textsubscript{i}) by using indo-1 in cultured rat vascular smooth muscle cells and cultured chick embryo ventricular myocytes. Exposure of cultured vascular smooth muscle cells to vasopressin (1-100 nM) dose-dependently produced an initial transient increase (from control level [Ca\textsuperscript{2+}]\textsubscript{i} of 133.6±10.9 nM to peak [Ca\textsuperscript{2+}]\textsubscript{i} of 842.7±172.8 nM at 100 nM vasopressin, p<0.01) and then a small sustained increase in [Ca\textsuperscript{2+}]\textsubscript{i}. After pretreatment of vascular smooth muscle cells with 1 \mu M OPC-21268, the effects of 100 nM vasopressin on [Ca\textsuperscript{2+}]\textsubscript{i} were abolished. Exposure of ventricular myocytes to 100 nM vasopressin slightly but significantly decreased peak systolic cell position (~8.7±3.7%, p<0.05) and also produced reductions in peak systolic [Ca\textsuperscript{2+}]\textsubscript{i}, (from 962.2±76.4 to 751.2±70.5 nM, p<0.01) within 30 seconds. Pretreatment of ventricular myocytes with OPC-21268 (1 \mu M) completely suppressed vasopressin-induced changes in peak systolic cell position and [Ca\textsuperscript{2+}]\textsubscript{i}. These results suggest that vasopressin may increase vascular tone and may also cause a direct negative inotropic effect via V\textsubscript{1} receptors and that this orally active V\textsubscript{1} antagonist (OPC-21268) may have potential clinical usefulness. (Hypertension 1992;19:730-733)

KEY WORDS • vasopressins • calcium • vascular smooth muscle • myocardium

Arginine vasopressin (AVP) is among the strongest vasoconstrictors, and plasma concentration of AVP is elevated in patients with congestive heart failure (CHF)\textsuperscript{1} and those with essential hypertension.\textsuperscript{2} The intracellular mechanisms underlying the vascular action of vasopressin (V\textsubscript{1} receptor) have been explained by the activation of phospholipase C and the subsequent mobilization of intracellular Ca\textsuperscript{2+} and activation of protein kinase C, identical with those of angiotensin II (Ang II). Inhibition of Ang II action by angiotensin converting enzyme inhibitors in hypertension and CHF have been proved to be effective.\textsuperscript{4} This suggests that V\textsubscript{1} antagonists may also have a beneficial effect in the treatment of hypertension and CHF. Many kinds of AVP antagonists were developed for therapeutic uses, all of which were peptide analogues and did not have oral bioavailability.\textsuperscript{5} Recently, an orally active, nonpeptide form of V\textsubscript{1} antagonist 1-[4(3-acetylamino)propoxy]benzoyl]-4-piperidyl)-3,4-dihydro-2(1H)-quinolinone (OPC-21268) has been randomly screened from thousands of synthetic compounds.\textsuperscript{6} The receptor pharmacology study has shown that OPC-21268 is highly selective for V\textsubscript{1} because the IC\textsubscript{50} for V\textsubscript{1} was 4.0×10\textsuperscript{-7} M, whereas the IC\textsubscript{50} for V\textsubscript{2} was more than 1.0×10\textsuperscript{-4} M.\textsuperscript{6}

In the present study, we observed simultaneously the effects of AVP and V\textsubscript{1} antagonist (OPC-21268) on cytosolic Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]\textsubscript{i}) in cultured rat vascular smooth muscle cells (VSMCs). We also investigated the effects of AVP and OPC-21268 on [Ca\textsuperscript{2+}]\textsubscript{i}, transients and cell contraction in cultured chick embryo ventricular myocytes since direct effects of AVP on [Ca\textsuperscript{2+}]\textsubscript{i}, and contractility in cardiac muscle are still controversial.\textsuperscript{7,9}

Methods

Culture of Vascular Smooth Muscle Cells and Ventricular Myocytes

VSMCs were cultured from the rat aorta (male Wistar rats; body weight, 200–300 g) by the explant method.\textsuperscript{10} The culture medium was composed of 90% RPMI 1640 medium (GIBCO Laboratories, Grand Island, N.Y.), 10% fetal calf serum (Cell Culture Laboratories, Cleveland, Ohio), and 0.1% penicillin-streptomycin antibiotics. The confluent cells after 7 days of culture were passaged and were again grown for 5 days on coverslips to be used for the assays described below. Layer cultures of spontaneously contracting ventricular myocardial cells were prepared from 10-day-old chick embryos by using the methods described elsewhere.\textsuperscript{11} Ventricular myocytes were isolated from minced ventricles with serial trypsinization and grown on coverslips in culture medium consisting of 6% heat-inactivated fetal calf serum, 40% medium 199 (GIBCO), 0.1% penicillin-streptomycin antibiotic solution, and 54% balanced salt...
solution containing (millimolar) NaCl 116, NaH2PO4 1.0, MgSO4 0.8, NaHCO3 26.2, CaCl2 0.9, and glucose 5. All studies were performed on ventricular cells after 3 days of culture.

Simultaneous Measurement of Cytosolic Ca2+ Concentration and Cell Motion

[Ca2+]i was measured with the Ca2+ fluorescent dye indo-1,12 as described by Peeters et al.13 Coverslips of cultured VSMCs or cultured chick ventricular cells were incubated at 37°C in culture medium containing 5 µM indo-1 AM (indo-1 acetoxymethyl ester; Dojin Kagaku, Kumamoto, Japan) for 15 minutes and then washed in indo-1-free solution for 30 minutes. After dye loading, a coverslip was placed in a flow-through cell chamber and continuously superfused with HEPES-buffered normal Tyrode’s solution containing (millimolar) NaCl 137.0, KCl 3.7, MgCl2 0.5, CaCl2 1.8, glucose 5.6, and HEPES (free acid) 4.0 titrated to pH 7.35 with 2.1 mM NaOH. The instrumentation used for fluorescence measurement has been described elsewhere.15-16 In the present work, [Ca2+]i was calibrated by the previously described method.14 Cell motion was measured by tracking the motion of a plastic microsphere attached to the surface of a layer of the cultured cells along a raster line segment of the video image.11 For normalization of cell motion, a 100% value was assigned to peak systolic cell position in the control condition and a 0% value to the end-diastolic cell position.16 Cultured ventricular myocytes were paced with field stimulation as described previously.15

Solutions

After the equilibrium period, the Tyrode’s solution containing AVP (Peptide Research Institute, Osaka, Japan) and/or OPC-21268 (Otsuka Vasopressin Antagonist Research Committee, Osaka, Japan) was superfused into the cell chamber. [Ca2+]i, and the cell motion of ventricular cells were stabilized within 2 minutes after exposure to test solutions, and then data were collected from an average of 10 consecutive beats.

Statistics

Data were expressed as mean±SEM. Paired Student’s t test or the Bonferroni method was used to compare the different groups. Values of p<0.05 were considered to be statistically significant.

Results

The effects of 1–100 nM AVP on [Ca2+]i in cultured adult rat VSMCs were studied. As shown in Figure 1 (upper panel), 100 nM AVP caused both a marked transient increase in [Ca2+]i (peak within 30 seconds) and a small but long-lasting sustained increase in [Ca2+]i. Each dose of AVP was tested sequentially, allowing the [Ca2+]i to return to baseline between doses (5-minute washout). Significant effects of AVP on peak [Ca2+]i were observed at a concentration as low as 1 nM (Table 1). Exposure to 1 µM OPC-21268 in the presence of 100 nM AVP decreased [Ca2+]i, to control levels (Figure 1, upper panel). Pretreatment of these cells with 1 µM OPC-21268 abolished the effects of AVP on [Ca2+]i (Figure 1, lower panel, and summarized in Table 1). On the other hand, exposure to 10 nM Ang II caused a prominent increase in [Ca2+]i in 1 µM OPC-21268–pretreated cells (data not shown).

Figure 2 (upper panel) exhibits representative tracings of [Ca2+]i, transients and motion before and after the addition of 100 nM AVP to the cultured chick embryo ventricular myocytes. Exposure to 100 nM AVP produced an initial transient increase and then a small sustained increase in [Ca2+]i. The subsequent exposure to 1 µM OPC-21268 returned the [Ca2+]i level to baseline. Lower panel: Effects of 100 nM AVP on [Ca2+]i in cultured rat vascular smooth muscle cells pretreated with 1 µM OPC-21268. [Ca2+]i was not changed at all after exposure to 100 nM AVP.

Table 1. Effects of Arginine Vasopressin and OPC-21268 on Cytosolic Ca2+ Concentration in Vascular Smooth Muscle Cells

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Peak [Ca2+]i (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>133.6±10.9</td>
</tr>
<tr>
<td>10^-4 M</td>
<td>224.1±41.0*</td>
</tr>
<tr>
<td>10^-5 M</td>
<td>391.6±65.0t</td>
</tr>
<tr>
<td>10^-6 M</td>
<td>842.7±172.8†</td>
</tr>
<tr>
<td>OPC-21268 treatment</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>146.5±25.0</td>
</tr>
<tr>
<td>10^-4 M</td>
<td>143.8±10.1</td>
</tr>
<tr>
<td>10^-4 M+AVP 10^-7 M</td>
<td>148.4±24.1</td>
</tr>
</tbody>
</table>

Values are mean±SEM. [Ca2+]i, cytosolic Ca2+ concentration; AVP, arginine vasopressin. *p<0.05, †p<0.01 vs. control. There were no significant differences among control, OPC-21268, and OPC-21268+AVP groups.
Hypertension Vol 19, No 6, Part 2 June 1992

... decreases in both peak systolic cell position and [Ca\(^{2+}\)], may be due to the increase in contractile frequency evoked by AVP, ventricular myocytes were paced constantly at 2.5 Hz. However, 100 nM AVP still decreased both peak systolic [Ca\(^{2+}\)] and cell position (Figure 2, lower panel). Exposure to 1 μM OPC-21268 itself did not change [Ca\(^{2+}\)], transients or cell motion. After pretreatment of ventricular cells with 1 μM OPC-21268, exposure to 100 nM AVP did not cause any significant changes in peak systolic [Ca\(^{2+}\)], and cell position or in beating frequency (Figure 3 and Table 2).

**Discussion**

In the present study, the newly synthesized AVP antagonist OPC-21268 completely inhibited the cellular effects of pharmacological doses of AVP in both VSMCs and ventricular myocytes. Although AVP-induced vasoconstriction is well understood, the direct cardiac effects of AVP do not seem to have been established. Intravenous infusions of AVP in conscious animals result in reduced cardiac output and coronary vascular constriction. However, it has been unclear if this decrease in cardiac output is a direct effect of AVP on myocardium or rather secondary to myocardial ischemia by coronary vasoconstriction. On the other hand, Walker et al. showed that low concentrations of AVP produced a direct positive inotropic effect in isolated perfused rat heart but that a high dose of AVP decreased contractility. They used a constant-flow perfused heart model, which is still not completely free from the effects of coronary vasoconstriction. We therefore studied the effects of relatively high doses of AVP and its antagonist on [Ca\(^{2+}\)], transients and myocardial contraction in cultured ventricular myocytes. As shown in Figure 2 (upper panel), 100 nM AVP slightly decreased peak systolic [Ca\(^{2+}\)], and cell position, but very recently it was reported that AVP increases [Ca\(^{2+}\)], in the neonatal rat ventricular cells via V1 receptors. The discrepancy between this report and ours may be explained by differences in species or the methods for [Ca\(^{2+}\)] measurements, i.e., beat-to-beat [Ca\(^{2+}\)] transients in the present study versus the time-averaged [Ca\(^{2+}\)]. Similarly, Ang II has been shown to decrease contractility and [Ca\(^{2+}\)], transients in the present study versus the time-averaged [Ca\(^{2+}\)].

![Figure 2](http://hyper.ahajournals.org/)

**FIGURE 2.** Upper panel: Representative tracings of arginine vasopressin (AVP)-induced changes in cytosolic Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) transients and cell contraction in spontaneously beating cultured chick embryo ventricular myocytes. Exposure to 100 nM AVP caused a decrease in peak systolic [Ca\(^{2+}\)], and cell position. Spontaneous rate of beats was increased after exposure to 100 nM AVP. Lower panel: Effects of 100 nM AVP on [Ca\(^{2+}\)], transients and cell motion in constant rate-paced (2.5 Hz) ventricular myocytes. Exposure of these cells to 100 nM AVP still decreased peak systolic [Ca\(^{2+}\)], and cell position within 60 seconds.

... after exposure to 100 nM AVP (131.4±10.6 versus 140.1±10.0 beats per minute, p<0.01). Superfusion of a vehicle solution did not cause any significant changes in the parameters measured throughout the study protocol. To exclude the possibility that the AVP-mediated decreases in both peak systolic cell position and [Ca\(^{2+}\)], may be due to the increase in contractile frequency evoked by AVP, ventricular myocytes were paced constantly at 2.5 Hz. However, 100 nM AVP still decreased both peak systolic [Ca\(^{2+}\)], and cell position (Figure 2, lower panel). Exposure to 1 μM OPC-21268 itself did not change [Ca\(^{2+}\)], transients or cell motion. After pretreatment of ventricular cells with 1 μM OPC-21268, exposure to 100 nM AVP did not cause any significant changes in peak systolic [Ca\(^{2+}\)], and cell position or in beating frequency (Figure 3 and Table 2).

**Table 2. Effects of Arginine Vasopressin and OPC-21268 on Cytosolic Ca\(^{2+}\) Concentration and Contraction in Ventricular Myocytes**

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Peak systolic [Ca(^{2+})], (nM)</th>
<th>Diastolic [Ca(^{2+})], (nM)</th>
<th>Peak systolic cell position (%)</th>
<th>Frequency of beating (bpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>962.2±76.4</td>
<td>216.2±16.4</td>
<td>100.0</td>
<td>131.4±10.6</td>
</tr>
<tr>
<td>10^{-7} M</td>
<td>751.2±70.5*</td>
<td>217.3±15.0††</td>
<td>91.3±3.7†</td>
<td>140.1±10.0††</td>
</tr>
<tr>
<td>OPC-21268</td>
<td>662.1±62.7</td>
<td>303.5±28.1</td>
<td>100.0</td>
<td>114.8±7.7</td>
</tr>
<tr>
<td>OPC-21268</td>
<td>636.0±101.0</td>
<td>297.8±15.2</td>
<td>99.2±2.5</td>
<td>114.4±9.3</td>
</tr>
<tr>
<td>OPC-21268+AVP</td>
<td>631.2±94.5</td>
<td>305.3±12.1</td>
<td>102.5±4.6</td>
<td>113.6±7.3</td>
</tr>
</tbody>
</table>

Values are mean±SEM. [Ca\(^{2+}\)], cytosolic Ca\(^{2+}\) concentration; bpm, beats per minute; AVP, arginine vasopressin. *p<0.01; †not statistically significant; ††p<0.05 vs. control. There were no significant differences among control, OPC-21268, and OPC-21268+AVP groups.
effects of AVP and Ang II may be observed only in cultured embryonic or neonatal ventricular cells.

The pathophysiological roles of AVP in cardiovascular disease are still controversial. Intravenous administration of antagonists for AVP has been known to rapidly decrease systemic vascular resistance in patients with elevated plasma AVP including hypertension and CHF. On the other hand, the administration of exogenous AVP did not always cause a substantial elevation of blood pressure when comparable plasma concentrations to those of patients with CHF or accelerated hypertension were obtained. Although recent studies try to explain this discrepancy by the central effects of AVP, which are synergistic with sympathetic nervous activity, further studies are required to clarify the roles of AVP. However, with this orally active antagonist for AVP, it becomes possible to examine its long-term effects and, in turn, to obtain a clue for the role of endogenous AVP. Furthermore, if so, this compound may have potential clinical usefulness.

Acknowledgments
We gratefully acknowledge Dr. E. Suzuki for supplying cultured vascular smooth muscle cells and Drs. S. Momomura, T. Sugimoto, and M. Iizuka for their support.

References
Effects of a nonpeptide vasopressin antagonist (OPC-21268) on cytosolic Ca$^{2+}$ concentration in vascular and cardiac myocytes.
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_Hypertension_. 1992;19:730-733
doi: 10.1161/01.HYP.19.6.730

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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