Effect of Propofol on Arachidonate Cascade by Vasopressin in Aortic Smooth Muscle Cells

Inhibition of PGI₂ Synthesis

Kumiko Tanabe, M.D.,* Osamu Kozawa, M.D., Ph.D.,† Hiroyuki Matsuno, Ph.D.,‡ Masayuki Niwa, Ph.D.,‡ Shuji Dohi, M.D., Ph.D.,§ Toshikiko Uematsu, M.D., Ph.D.||

Background: The mechanisms underlying the vascular effects of propofol are not fully understood. Vasopressin, a potent vasoactive peptide, stimulates the arachidonate cascade and the synthesis of prostacyclin (PGI₂; the main metabolite of the cascade in vascular smooth muscle cells). Arachidonic acid (AA) release by phospholipases is the rate-limiting step in the cascade. We investigated the mechanisms underlying vasopressin-induced AA release and the effect of propofol on PGI₂ synthesis in a rat aortic smooth muscle cell line: A10 cells.

Methods: In cultured A10 cells pretreated with propofol, the stimulation by vasopressin of AA release and PGI₂ synthesis was evaluated by measuring 3H]AA and 6-keto PGF₁α, respectively, in the culture medium. The effects of propofol on vasopressin-induced activation of phosphoinositide-hydrolyzing phospholipase C and phosphatidylycholine-hydrolyzing phospholipase D were evaluated by measuring inositol phosphate formation and choline formation, respectively.

Results: A phospholipase C inhibitor and a phosphatidic acid phosphohydrolase inhibitor both attenuated vasopressin-induced AA release and PGI₂ synthesis, as did a phospholipase A₂ inhibitor. Propofol inhibited vasopressin-induced activation of phosphoinositide-hydrolyzing phospholipase C and phosphatidylycholine-hydrolyzing phospholipase D, but this effect of propofol was significant only at supraclinical concentration (0.1 µM). Propofol reduced vasopressin-induced PGI₂ synthesis. The inhibitory effect was observed at concentrations (10 µM–0.1 µM) higher than those used clinically.

Conclusions: Propofol suppresses the arachidonate cascade caused by vasopressin at least partly by inhibiting phosphoinositide-hydrolyzing phospholipase C and phosphatidylycholine-hydrolyzing phospholipase D, resulting in the inhibition of PGI₂ synthesis. Propofol-mediated inhibition of vasopressin-stimulated synthesis of PGI₂ may reduce the vasorelaxation by propofol. (Key words: A10 cells; arachidonic acid; phospholipase; propofol; vasopressin.)

PROPOFOL (2,6-diisopropylphenol) is commonly used for inducing and maintaining general anesthesia. It causes cardiovascular changes in vivo, and it has been reported to have direct effects on both the myocardium and the peripheral vasculature. In addition, propofol has indirect effects on the cardiovascular system through its effects on sympathetic activity, baroreflex activity, and central nervous system activity. In a previous study, we showed that propofol inhibits endothelin-1-induced Ca²⁺ mobilization and protein kinase C activation in a cultured vascular smooth muscle cell line (A10 cells) derived from rat aortic smooth muscle cells. We speculated that propofol might have vascular effects via direct effects on the vascular smooth muscle cells.

Vascular smooth muscle cells play important roles in the regulation of vascular tone. Vasopressin is a potent vasoactive peptide, and it has been reported to bind to V₁ receptors in vascular smooth muscle cells, including A10 cells. Vasopressin induces vasoconstriction through mobilization of Ca²⁺ and protein kinase C activation in vascular smooth muscle cells. Vasopressin has been shown to stimulate phosphoinositide hydrolysis by phospholipase C and also induce phosphatidylycholine breakdown by phospholipase D as well as endothelin-1 in vascular smooth muscle cells.

Vasopressin also stimulates the synthesis of prostaglandins such as prostacyclin (PGI₂), a potent vascular relaxing agent and it is the main metabolite of arachidonic acid (AA) in basal and vasopressin-stimu-
lated vascular smooth muscle cells. Arachidonic acid release is the rate-limiting step in the arachidonate cascade. It is generally recognized that phospholipase A2 releases AA directly from membrane stores of esterified phospholipids. Vaspressin-induced vasoconstriction may be self-modulated by vaspressin-stimulated PGII synthesis. Vaspressin also directly activates phospholipase A2, thus releasing AA in vascular smooth muscle cells. Arachidonic acid also is released from phospholipids by other phospholipases such as phospholipase C and phospholipase D, however, the exact mechanism underlying vaspressin-induced AA release has not yet been elucidated fully in vascular smooth muscle cells.

There has been no report showing the effect of propofol on the arachidonate cascade in vascular smooth muscle cells. We previously reported that propofol inhibits endothelin-1-induced phosphoinositiode–phospholipase C and phosphatidylycholine–phospholipase D activity in A10 cells. Our hypothesis was that propofol might also inhibit vaspressin-induced phosphoinositiode–phospholipase C, phosphatidylycholine–phospholipase D and phospholipase A2 activity, resulting in suppression of the arachidonate cascade. Therefore, we first investigated whether these phospholipases are involved in vaspressin-induced AA release in cultured A10 cells. Then, we evaluated the effects of propofol on vaspressin-induced phosphoinositiode and phosphatidylycholine hydrolysis and PGII (main metabolite of AA) synthesis in these cells.

Materials and Methods

**Materials**

- [5,6,8,9,11,12,14,15]HAA (208 Ci/mmol), myo-[3H]-inositol (81.5 Ci/mmol), [methyl-3H]choline chloride (85 Ci/mmol), and a 6-keto PGF1α radioimmunoassay kit were obtained from Amersham Japan (Tokyo, Japan). Arginine vaspressin was purchased from Peptide Institute (Minoh, Japan). 1-(6c(17β,3-Methoxyestradiol, 1, 3, 5, 10-trien-17-ylamino)hexyl)-1H-pyrrole-2,5-dione (U-73122), a phospholipase C inhibitor, was purchased from Funakoshi Pharmaceutical (Tokyo, Japan). D,L-Propranolol hydrochloride (propranolol), a phosphatidic acid phosphohydrolase inhibitor, was purchased from Wako Pure Chemical (Osaka, Japan). Propofol was purchased from Aldrich (Tokyo, Japan). Essentially fatty acid-free bovine serum albumin (BSA) and quinacrine, a phospholipase A2 inhibitor, were purchased from Sigma Chemical (St. Louis, MO). Other materials and chemicals were obtained from commercial sources.

Vaspressin was dissolved in an assay buffer (consisting of 5 mm HEPES, pH 7.4; 150 mm NaCl; 5 mm KCl; 0.8 mm MgSO4; 1 mm CaCl2; and 5.5 mm glucose) containing 0.01% BSA. We used this BSA-containing assay buffer as the vehicle control for vaspressin. U-73122, propranolol, and quinacrine were each dissolved in dimethyl sulfoxide. Propofol was dissolved in ethanol. The maximum concentration of ethanol or dimethyl sulfoxide in the culture medium was 0.1%, and this did not affect the detection of AA release, measurement of PGII synthesis, or the formation of inositol phosphates and choline. We used assay buffer containing 0.01% BSA and 0.1% dimethyl sulfoxide as the vehicle control for U-73122, propranolol, and quinacrine. We used assay buffer containing 0.01% BSA and 0.1% ethanol as the vehicle control for propofol.

**Cell Culture**

A10 cells were obtained from the American Type Culture Collection (Rockville, MD). The cells (1 × 10⁵) were seeded into 35-mm-diameter dishes and maintained at 37°C in a humidified atmosphere of 5% carbon dioxide and 95% air in 2 ml of Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum. After 5 days, the medium was exchanged for 2 ml of serum-free Dulbecco’s modified Eagle’s medium. The cells were used for experiments 48 h thereafter. For measurements of the formation of inositol phosphates, the medium was exchanged for 2 ml of inositol-free Dulbecco’s modified Eagle’s medium.

**Assay for AA Release**

As previously described, cultured cells were labeled with [3H]AA (0.5 µCi/dish) for 48 h. The labeled cells were washed four times with 1 ml of the assay buffer and subsequently preincubated in 1 ml of assay buffer containing 0.1% essentially fatty acid-free BSA at 37°C for 20 min. The cells were then stimulated by various doses of vaspressin. After 30 min, the medium was collected and its radioactivity was determined. To determine whether activation of phosphoinositiode-hydrolyzing phospholipase C, phosphatidylycholine-hydrolyzing phospholipase D, or phospholipase A2, are involved in vaspressin-induced AA release and PGII synthesis in A10 cells, we evaluated the effects of U-73122, propranolol, or quinacrine, respectively, on vaspressin-induced AA release and PGII synthesis. Pretreatment with U-73122
PROPOFOL'S EFFECTS ON AORTIC SMOOTH MUSCLE CELLS

(0.3–10 μM), propranolol (5–300 μM), quinacrine (5–30 μM), or vehicle was performed for 20 min.

Measurement of PGI₂ Synthesis

As previously described,25 PGI₂ synthesis was determined using methods similar to those described in the section "assay for AA release," except that unlabeled cells were used. Cultured cells were pretreated with U-73122 (0.3–10 μM), propranolol (5–300 μM), quinacrine (5–30 μM), propofol (0.1 μM–0.1 mm), or vehicle for 20 min and then stimulated by various doses of vasopressin. After the indicated period, the medium was collected, and the level of 6-keto PGF₁α (a stable and inactive metabolite of PGI₂)27,34 in the medium was determined by means of an radioimmunoassay kit. Briefly, an assay buffer (0.05 M phosphate buffer, pH 7.3, containing 0.05% BSA and 0.1% sodium azide), the medium, the 6-keto PGF₁α tracer (6-keto PGF₁α [125]iodotyrosine methyl ester in ethanol and water) and the 6-keto PGF₁α antiserum were transferred to tubes to produce a final ratio 2:1:1:1. The contents were mixed and then incubated over 15 h at 4°C. Amerlex™-M second antibody reagent was added to the tubes. The tubes were centrifuged at 14,000g for 10 min, and the supernatants were discarded. Then, the radioactivity was determined. The value of the samples were calculated from the standard curve. The cross-reactivity between 6-keto PGF₁α and other prostaglandins was less than 1%.

Formation of Inositol Phosphates

As previously described,35,36 cultured cells were labeled with [methyl-³H]inositol (3 µCi/dish) for 48 h. The labeled cells were preincubated with 20 mm LiCl for 10 min at 37°C in 1 ml of assay buffer containing 0.01% BSA. The cells were stimulated by vasopressin for 20 min after pretreatment of U-73122 or 150 min after pretreatment of propofol. The reaction was discontinued by adding 1 ml trichloroacetic acid, 30%. The acidic supernatant was treated with diethyl ether to remove the acid and then neutralized with 0.1 N NaOH. The supernatant was applied to an anion exchange column containing 1 ml of Dowex AG1-X8 (100–200 mesh, formate form; Bio-Rad Laboratories, Hercules, CA). The radioactive inositol phosphates were eluted with 8 ml formic acid, 0.1 M, containing 1 M ammonium formate.35,36 Pretreatment with propofol (0.1 μM–0.1 mm), U-73122 (0.3–10 μM), or vehicle was performed for 20 min.

Choline Formation

As previously described,37 to help determine the phosphatidylcholine-hydrolyzing phospholipase D activity in A10 cells, cultured cells were labeled with [methyl-³H]choline chloride (3 µCi/dish) for 48 h. The labeled cells were washed twice with 1 ml of the assay buffer and then pretreated with propofol (0.1 μM–0.1 mm) or vehicle for 20 min at 37°C in 1 ml of assay buffer containing 0.01% BSA. They were then stimulated by vasopressin for 150 min until the reaction was discontinued by adding 0.75 ml of ice-cold methanol. The dishes were then placed on ice for 30 min, and the contents were transferred subsequently to tubes to which chloroform was added. They were then left standing on ice for 60 min, whereupon chloroform and water were added to produce a final ratio of 1:1:0.9 (chloroform:methanol:water). The tubes were centrifuged at 14,000g for 5 min, and the upper aqueous methanolic phase was taken for analysis of the water-soluble choline-containing metabolites. Separation was conducted on a column containing 1 ml of Dowex 50-WH⁺ (Bio-Rad Laboratories, 200–400 mesh, Hercules, CA) as described,37 with a minor modification.15 Briefly, the phase was diluted to 5 ml with water and applied to the column. Glycerophosphocholine and choline phosphate were removed with 24 ml of water, and radioactive choline was then eluted with 10 ml HCl, 1 M.

Determination

The radioactivity of ³H samples was determined using a Beckman LS6500C liquid scintillation spectrometer (Fullerton, CA). The radioactivity of ¹²⁵I samples was determined using a Wallac 1480 WIZARD 3¹ automatic gamma counter (Turku, Finland).

Statistical Analysis

The data were analyzed by one-way analysis of variance, followed by the Bonferroni correction for multiple comparisons between pairs. Probability values < 0.05 were considered significant. All data are presented as the mean ± SD of triplicate determinations.

Results

Effect of Vasopressin on AA Release and PGI₂ Synthesis

It has been reported that vasopressin stimulates AA release in a time- and dose-dependent manner in A10 cells.36 We confirmed that vasopressin significantly in-
synthesis in a time-dependent manner (up to 180 min) in A10 cells (fig. 2A). Significant 6-keto PGF₁₀合成 occurred when vasopressin-stimulation was continued for 120 min or more. The stimulatory effect of vasopressin on 6-keto PGF₁₀ synthesis was dose-dependent over the range 0.1 nM to 0.1 μM (fig. 2B), the maximum effect being observed at 10 nM.

Effect of U-73122 on Vasopressin-induced AA Release and PGI₂ Synthesis

It has been reported that vasopressin induces phospholipase C-catalyzed phosphoinositide hydrolysis in A10 cells and that phosphoinositide hydrolysis results in the formation of diacylglycerol and inositol phosphates. First, we confirmed that vasopressin induces the formation of inositol phosphates in these cells (fig. 3). Vasopressin induced the formation of inositol phosphates in a time-dependent manner (up to 150 min). U-73122 is known to be a potent inhibitor of phosphoinositide-hydrolyzing phospholipase C. To establish whether activation of phosphoinositide-hydrolyzing phospholipase C is involved in the stimulation of AA release by vasopressin in A10 cells, we evaluated the effect of U-73122 on vasopressin-induced AA release. Pretreatment with 3 μM U-73122, which by itself had little or no effect on AA release, significantly inhibited vasopressin (0.1 μM)-induced AA release (table 1).

We also found that pretreatment with U-73122, which by itself had little effect on the formation of inositol phosphates, significantly inhibited the vasopressin (0.1 μM)-induced formation of inositol phosphates in A10 cells. This inhibitory effect of U-73122 (10 μM) was
PROPOFOL'S EFFECTS ON AORTIC SMOOTH MUSCLE CELLS

Fig. 3. Effect of propofol on vasopressin-induced formation of inositol phosphates in A10 cells. ([H]inositol-labeled cells were pretreated with various doses of propofol for 20 min, then stimulated with 0.1 μM vasopressin (●) or vehicle (○) for 150 min. The formation of inositol phosphates was then determined. Each value represents the mean ± SD of triplicate determinations in a single experiment (representative of three experiments in all). *P < 0.05 versus the value for vasopressin without propofol pretreatment. The arrow indicates the formation of inositol phosphates of the cells pretreated in the absence of propofol.

significant and dose-dependent over the range 1 to 10 μM, the maximum effect being observed at 10 μM (table 2).

U-73122, at a dose that by itself had little effect on 6-keto PGF₁α synthesis, significantly reduced vasopressin (0.1 μM)-induced 6-keto PGF₁α synthesis (10.0 ± 0.27 ng/dish for 0.1 μM vasopressin with vehicle pre-

Table 1. Effect of U-73122 on Vasopressin-induced Arachidonic Acid (AA) Release in A10 Cells

<table>
<thead>
<tr>
<th></th>
<th>AA Release (cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1,328 ± 72</td>
</tr>
<tr>
<td>Vasopressin</td>
<td>2,696 ± 78</td>
</tr>
<tr>
<td>U-73122 (0.3 μM)</td>
<td>1,274 ± 478</td>
</tr>
<tr>
<td>U-73122 (0.3 μM) + vasopressin</td>
<td>2,312 ± 214</td>
</tr>
<tr>
<td>U-73122 (3 μM)</td>
<td>1,314 ± 538</td>
</tr>
<tr>
<td>U-73122 (3 μM) + vasopressin</td>
<td>1,610 ± 234</td>
</tr>
</tbody>
</table>

Table 2. Effect of U-73122 on Vasopressin-induced Inositol Phosphates Formation in A10 Cells

<table>
<thead>
<tr>
<th></th>
<th>Inositol Phosphates Formation (cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>1,670 ± 288</td>
</tr>
<tr>
<td>vasopressin</td>
<td>7,013 ± 1,052</td>
</tr>
<tr>
<td>U-73122 (10 μM)</td>
<td>1,635 ± 171</td>
</tr>
<tr>
<td>U-73122 (10 μM) + vasopressin</td>
<td>3,826 ± 812*</td>
</tr>
</tbody>
</table>

[U-73122 pretreated cells were pretreated with U-73122 or vehicle for 20 min, then stimulated by 0.1 μM vasopressin for 20 min. Each value represents the mean ± SD of triplicate determinations in a single experiment (representative of three experiments in all). *P < 0.05 versus the value for vasopressin without U-73122 pretreatment.

Effect of Propranolol on Vasopressin-induced AA Release and PGI₂ Synthesis

It has been reported that vasopressin induces phospholipase D-catalyzed phosphatidylcholine hydrolysis in A10 cells33 and that phosphatidylcholine hydrolysis by phospholipase D results in the formation of phosphatic acid and choline.31-43 First, we confirmed that vasopressin induces the formation of choline in these cells (23,503 ± 1,245 cpm for vehicle; 35,140 ± 1,087** cpm for 0.1 μM vasopressin). Vasopressin (0.1 μM) induced the formation of choline in a time-dependent manner (up to 150 min). To clarify whether activation of phosphatidylcholine-hydrolyzing phospholipase D is involved in the stimulation of AA release and PGI₂ synthesis by vasopressin in A10 cells, we evaluated the effect of propranolol (an inhibitor of phosphatidic acid phosphohydrolase,31,52 which converts phosphatidic acid to diacylglycerol31-45) on vasopressin-induced AA release and 6-keto PGF₁α synthesis. Pretreatment with 300 μM propranolol, which by itself had little or no effect on AA release, significantly inhibited the vasopressin (0.1 μM)-induced AA release (table 3). In addition, propranolol, at doses that by themselves had no detectable effect on 6-keto PGF₁α synthesis, significantly reduced vasopressin (0.1 μM)-induced 6-keto PGF₁α synthesis in a dose-dependent manner (fig. 4).

* P < 0.05 versus the value for vasopressin without U-73122 pretreatment.
** P < 0.05 versus the value for vehicle.

Anesthesiology, V 90, No 1, Jan 1999
Table 3. Effect of Propranolol on Vasopressin-induced Arachidonic Acid (AA) Release in A10 Cells

<table>
<thead>
<tr>
<th></th>
<th>AA Release (cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1,146 ± 90</td>
</tr>
<tr>
<td>Vasopressin</td>
<td>2,563 ± 159</td>
</tr>
<tr>
<td>Propranolol (50 µM)</td>
<td>1,014 ± 256</td>
</tr>
<tr>
<td>Propranolol (50 µM) + vasopressin</td>
<td>1,811 ± 163</td>
</tr>
<tr>
<td>Propranolol (300 µM)</td>
<td>1,109 ± 105</td>
</tr>
<tr>
<td>Propranolol (300 µM) + vasopressin</td>
<td>1,129 ± 436*</td>
</tr>
</tbody>
</table>

[1] VAA-labeled cells were pretreated with propranolol or vehicle for 20 min, then stimulated by 0.1 µM vasopressin for 30 min.
Each value represents the mean ± SD of triplicate determinations in a single experiment (representative of three experiments in all).

* P < 0.05 versus the value for vasopressin without propranolol pretreatment.

Effect of Propofol on Vasopressin-induced Formation of Inositol Phosphates

We reported previously that propofol inhibits the formation of inositol phosphates that is induced by endothelin-1 in A10 cells.15 In the current study, pretreatment with propofol, which by itself had no detectable effect on inositol phosphates formation, inhibited the vasopressin (0.1 µM)-induced formation of inositol phosphates. The only significant effect of propofol on inositol phosphates formation was seen at a dose of 0.1 mM (a 40% reduction in the effect of vasopressin, fig. 3).

Effect of Propofol on the Vasopressin-induced Formation of Choline

Pretreatment with propofol, which by itself had little effect on the formation of choline, significantly inhibited the vasopressin (0.1 µM)-induced formation of choline. At 0.1 mM, propofol reduced the vasopressin (0.1 µM)-induced choline formation by approximately 40% (11,560 ± 2,671 cpm for 0.1 µM vasopressin with vehicle pretreatment; 4,180 ± 2,898† cpm for 0.1 µM vasopressin with 0.1 mM propofol pretreatment, values for vasopressin-unstimulated cells were subtracted to produce each data point).

Effect of Propofol on the PGI₂ Synthesis Induced by Vasopressin

Pretreatment with propofol (10 µM), which by itself had no detectable effect on 6-keto PGF₁α synthesis, significantly attenuated the time-dependent (up to 180 min) stimulation by vasopressin (0.1 µM) of 6-keto PGF₁α synthesis (fig. 2A). The effect of propofol on 6-keto PGF₁α synthesis was significant at doses more than 10 µM (fig. 5).

Effect of Quinacrine on Vasopressin-induced AA Release and PGI₂ Synthesis

It has been reported that phospholipase A₂ activation is involved in vasopressin-induced AA release in A10 cells.28 We confirmed that quinacrine, known to be a phospholipase A₂ inhibitor,35 at a dose that by itself had little effect on AA release or 6-keto PGF₁α synthesis, significantly inhibited the vasopressin-induced AA release (data not shown) and 6-keto PGF₁α synthesis (10.7 ± 0.37 ng/dish for 0.1 µM vasopressin with vehicle pretreatment; 6.3 ± 1.1†† ng/dish for 0.1 µM vasopressin with 30 µM quinacrine pretreatment, values for vasopressin-unstimulated cells were subtracted to produce each data point).

Discussion

In the current study, we demonstrated that vasopressin-induced AA release was dose-dependently reduced...
PROPOFOL’S EFFECTS ON AORTIC SMOOTH MUSCLE CELLS

Fig. 5. Effect of propofol on vasopressin-induced 6-keto PGF₁α synthesis in A10 cells. Cultured cells were pretreated with various doses of propofol for 20 min, then stimulated with 0.1 μM vasopressin (○) or vehicle (●) for 150 min. Each value represents the mean ± SD of triplicate determinations in a single experiment (representative of three experiments in all). *P < 0.05 versus the value for vasopressin without propofol pretreatment. The arrow indicates the 6-keto PGF₁α synthesis of the cells pretreated in the absence of propofol.

by U-73122, an inhibitor of phosphoinositide-hydrolyzing phospholipase C,⁴⁰ in a rat aortic smooth muscle cell line: A10 cells. It has been reported that vasopressin induces phosphoinositide-hydrolysis, resulting in the formation of diaclylglycerol and inositol phosphates.²¹,⁴⁰ Diacylglycerol and inositol 1,4,5-trisphosphate then serve as messengers for the activation of protein kinase C and the mobilization of intracellular Ca²⁺, respectively.²⁰,²¹,⁴⁰ In addition, diacylglycerol, which is a source of AA, is converted to monoacylglycerol and fatty acids in A10 cells.⁴¹ Thus, our findings suggest that phosphoinositide hydrolysis by phospholipase C is involved in the stimulation of AA release by vasopressin in these cells. We next showed that propranolol, an inhibitor of phosphatidic acid phosphohydrolase,³¹,³² also significantly inhibited vasopressin-induced AA release in A10 cells. It has been reported that vasopressin stimulates phosphatidylycholine-hydrolyzing phospholipase D in A10 cells,²²,²₃ in which phosphatidylycholine breakdown is downstream from phosphoinositide-hydrolysis by phospholipase C.²⁵ Phosphatidylycholine is hydrolyzed by phospholipase D, resulting in the formation of choline and phosphatidic acid, which is further degraded to diacylglycerol by phosphatidic acid phosphohydrolase.⁴¹-⁴⁵ Therefore, to judge from our results, the conversion of phosphatidic acid to diacylglycerol is involved in vasopressin-induced AA release in A10 cells. It has been reported that phospholipase A₂ plays an important role in vasopressin-induced AA release in A10 cells.²⁸ We confirmed that quinacrine, a phospholipase A₂ inhibitor, suppression vasopressin-induced AA release in these cells. On the basis of our current findings, we suggest that phosphoinositide-phospholipase C and phosphatidylycholine-phospholipase D (and phospholipase A₂) are involved in vasopressin-induced AA release in A10 cells.

PGI₂ is well known to be a major eicosanoid product derived from AA in vascular smooth muscle cells.²⁷ In the current study, we demonstrated that vasopressin stimulates PGI₂ synthesis in A10 cells and that U-73122 and propranolol both significantly reduced this vasopressin-induced PGI₂ synthesis, and AA release, in these cells. In addition, we confirmed that quinacrine inhibited vasopressin-induced PGI₂ synthesis in A10 cells. Our findings, therefore, strongly suggest that phosphoinositide hydrolysis by phospholipase C and phosphatidylycholine hydrolysis by phospholipase D are involved in the mechanism by which vasopressin induces PGI₂ synthesis in A10 cells.

In the current study, we showed that propofol suppressed vasopressin-induced PGI₂ synthesis in A10 cells. In these cells, it has been reported that the activation of phosphatidylycholine-hydrolyzing phospholipase D by vasopressin is kinetically downstream from the initial phosphoinositide hydrolysis by phospholipase C, and that it involves the intermediate activation of protein kinase C.²⁵ We demonstrated that propofol suppressed the vasopressin-induced formation of inositol phosphates and choline in these cells. We reported previously¹⁵ that propofol suppresses the endothelin-1-induced formation of inositol phosphates and choline in A10 cells. Our current findings suggest that propofol suppresses the stimulation by vasopressin of the arachidonic cascade, at least partly, by inhibiting phosphoinositide-hydrolyzing phospholipase C and phosphatidylycholine-hydrolyzing phospholipase D in A10 cells. However, the inhibition by propofol of the activation of phosphoinositide-hydrolyzing phospholipase C or phosphatidylycholine-hydrolyzing phospholipase D was partial. In contrast, we showed that 0.1 μM propofol suppressed vasopressin-induced PGI₂ synthesis almost completely. Therefore, it is likely that an inhibition of phospholipase A₂ is also involved in the suppression of
PGI₂ synthesis by propofol. The effects of propofol on intracellular signaling system in vascular smooth muscle cells shown in our current and in previous studies are shown in figure 6.

The vascular action of propofol has been reported to be caused by its direct effects on endothelial cells and vascular smooth muscle cells and by indirect effects such as those exerted via the central nervous system. We have shown, using the A10 cell line, that propofol suppresses the intracellular signaling responsible for the contraction of vascular smooth muscle. Because the current results clearly indicate that propofol also inhibits vasopressin-induced PGI₂ synthesis, it can be assumed that propofol will modulate the effects of vasodilators and of vasoconstrictors. The vasoconstrictive effect of vasopressin may be mediated by diacylglycerol and inositol trisphosphate produced by phospholipases C and D, and this vasoconstrictive effect of vasopressin is self-modulated by subsequent release of PGI₂. The vasorelaxing effect of propofol may in part be explained by its inhibition of vasopressin-mediated activation of phospholipase C and phospholipase D. Such relaxation may partially be offset by the simultaneous effect of propofol to attenuate vasopressin-stimulated PGI₂ synthesis. Mobilization of cytosolic Ca²⁺ and sustained protein kinase C activation induced by phosphoinositide-phospholipase C and phosphatidylinositol-phospholipase D have crucial roles in the induction of vasoconstriction. However, AA release and 6-keto-PGF₁α synthesis means PGI₂ synthesis in cultured aortic smooth muscle cells does not occur immediately after stimulation. In the current study, significant 6-keto-PGF₁α production began at 120 min after vasopressin-stimulation in A10 cells. Clinically, propofol is used not only for induction of anesthesia, but also for maintenance by continuous intravenous administration during several hours. In the current study, propofol-treatment was continued for 170 min. Therefore, it is possible that the suppression of vasopressin-induced PGI₂ synthesis by propofol has a role in automodulating the propofol-induced vasodilation during long-time anesthesia rather than during bolus infusion.

The plasma concentration of propofol recently has been reported to be 56–190 µg/ml in patients in whom general anesthesia was maintained with continuous intravenous infusion of propofol alone. In addition, it has been shown that approximately 97% of propofol is bound to plasma proteins. Therefore, free concentration of propofol is estimated to be 2–6 µg/ml. The inhibitory effect of propofol in the current study was observed at concentrations higher than those used clinically. However, the concentration of vasopressin that we used far exceeds in vivo values. Therefore, it is possible that the need for high concentrations of vasopressin and propofol in vivo may be because of its condition compared with in vivo conditions.

In conclusion, these results suggest that propofol suppresses the stimulation of the arachidonate cascade by vasopressin at least partly by inhibiting phosphoinositide-phospholipase C and phosphatidylinositol-phospholipase D. Propofol-mediated inhibition of vasopressin-stimulated synthesis of PGI₂ may reduce the vasoconstriction by propofol.
References

10. Chang KSK, Davis RF: Propofol produces endothelium-independent vasodilation and may act as a Ca2+ channel blocker. Anesth Analg 1993; 76:24–32


