Vasopressin-stimulated Ca\textsuperscript{2+} spiking in vascular smooth muscle cells involves phospholipase D

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Li, Yanxia, Aaron J. Shiel, Gary Maszak, and Kenneth L. Byron. Vasopressin-stimulated Ca\textsuperscript{2+} spiking in vascular smooth muscle cells involves phospholipase D. Am J Physiol Heart Circ Physiol 280: H2658–H2664, 2001.—Physiological concentrations of [Arg\textsuperscript{8}]vasopressin (AVP; 10–500 pM) stimulate oscillations of cytosolic free Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]\textsubscript{i}) in A7r5 vascular smooth muscle cells. We previously reported that this effect of AVP was blocked by a putative phospholipase A\textsubscript{2} (PLA\textsubscript{2}) inhibitor, ONO-RS-082 (5 \textmu M). In the present study, the products of PLA\textsubscript{2}, arachidonic acid (AA), and lysophospholipids were found to be ineffective in stimulating Ca\textsuperscript{2+} spiking, and inhibitors of AA metabolism did not prevent AVP-stimulated Ca\textsuperscript{2+} spiking. Thin layer chromatography was used to monitor the release of AA and phosphatidic acid (PA), which are the products of PLA\textsubscript{2} and phospholipase D (PLD), respectively. AVP (100 pM) stimulated both AA and PA formation, but only AA formation was inhibited by ONO-RS-082 (5 \textmu M). Exogenous PLD (type VII; 2.5 U/ml) stimulated Ca\textsuperscript{2+} spiking equivalent to the effect of 100 pM AVP. AVP stimulated transphosphatidylolysis of 1-butanol (a PLD-catalyzed reaction) but not 2-butanol, and 1-butanol (but not 2-butanol) completely prevented AVP-stimulated Ca\textsuperscript{2+} spiking. Protein kinase C (PKC) inhibition, which completely prevents AVP-stimulated Ca\textsuperscript{2+} spiking, did not inhibit AVP-stimulated phosphatidylbutanol formation. These results suggest that AVP-stimulated Ca\textsuperscript{2+} spiking depends on activation of PLD rather than PLA\textsubscript{2} and that PKC activation may be downstream of PLD in the signaling cascade.

A7r5; lipid hydrolysis; protein kinase C; signal transduction

Ca\textsuperscript{2+} spikes arise due to Ca\textsuperscript{2+} spiking from intracellular Ca\textsuperscript{2+} stores. The concentration of AVP needed to half-maximally increase [Ca\textsuperscript{2+}]\textsubscript{i} and release intracellular Ca\textsuperscript{2+} (\sim 2 nM) is much higher than the vasoconstrictor concentrations of AVP normally found in the systemic circulation (10–100 pM). This raises a question as to whether the release of intracellular Ca\textsuperscript{2+} stores can account for the vasoconstrictor effects of AVP. We have recently shown (2) that physiological concentrations of AVP (10–500 pM) stimulate oscillations of [Ca\textsuperscript{2+}]\textsubscript{i} (Ca\textsuperscript{2+} spikes) that increase in frequency with increasing AVP concentration ([AVP]) in A7r5 vascular smooth muscle cells. These Ca\textsuperscript{2+} spikes arise due to Ca\textsuperscript{2+}-dependent action potentials. Hence, in contrast to InsP\textsubscript{3}-mediated release of intracellular Ca\textsuperscript{2+}, the Ca\textsuperscript{2+} spikes have a strict requirement for extracellular Ca\textsuperscript{2+} and are abolished by blockers of voltage-sensitive Ca\textsuperscript{2+} channels.

AVP-stimulated Ca\textsuperscript{2+} spiking in A7r5 cells was previously found (2) to be blocked by a putative inhibitor of phospholipase A\textsubscript{2} (PLA\textsubscript{2}), ONO-RS-082, leading to the conclusion that PLA\textsubscript{2} may mediate this effect of AVP. The present study examines in more detail the lipid products of PLA\textsubscript{2} and the action of ONO-RS-082. The results suggest that phospholipase D (PLD) rather than PLA\textsubscript{2} may mediate the stimulation of Ca\textsuperscript{2+} spiking by AVP.

MATERIALS AND METHODS

Cell culture. A7r5 cells were cultured as described previously (3). Cells were subcultured onto rectangular 9 \times 22-mm (no. 1 1⁄2) glass coverslips or plastic tissue-culture dishes (Corning). Confluent cells (passages 10–30) were used 2–5 days after plating. The health and phenotype of the cells were verified routinely by examining [Ca\textsuperscript{2+}]\textsubscript{i}, responses to 100 pM AVP. Similar responses were obtained with all cell passages tested including those used for all of the biochemical assays.

Loading cells with fura 2. Coverslips were washed twice with control medium (in mM: 135 NaCl, 5.9 KCl, 1.5 CaCl\textsubscript{2}, 1.2 MgCl\textsubscript{2}, 11.5 glucose, and 11.6 HEPES/NaOH, pH 7.3) and then incubated in the same medium with 2 \textmu M fura 2-acetoxyethyl ester (AM), 0.1% bovine serum albumin (BSA), and 0.025% Pluronic F-127 detergent (20) for 90–120 min at room temperature (20–23°C). After loading, the cells were washed twice and incubated in control medium for 1–5 h before the start of the experiment. This final incubation allowed for complete hydrolysis of fura 2-AM as assessed by the shift in the fluorescence spectrum (24). As noted previously (3), this dye-loading protocol appeared not to adversely affect the cells. About 95% of the fura 2 was released from the...
cells within 3 min of saponin (50 μg/ml) addition which suggests that ~95% of the dye was in the cytosol.

\[ [\text{Ca}^{2+}]_i \] measurements. Fura 2 fluorescence was measured in cell populations with a Perkin-Elmer LS50B fluorescence spectrophotometer. This instrument is equipped with a rotating filter wheel that can be used to alternate 340 and 380 nm excitation at approximately 50 Hz. A coverslip was mounted vertically at a 30° angle to the light path in a cuvette that was continuously perfused with media. A four-way valve mounted just above the cuvette allowed for rapid switching of solutions; replacement of the medium bathing the cells has a half-time of ~20 s. The excitation light illuminated an area of ~30 mm² on the coverslip for recording of fluorescence from several thousand cells. Background fluorescence was determined at the end of the experiment by quenching the fura 2 fluorescence for 10–15 min in the presence of 5 μM ionomycin and 6 mM MnCl₂ in Ca²⁺-free medium. After background fluorescence was subtracted, the 340- and 380-nm value ratios were calculated and calibrated in terms of \([\text{Ca}^{2+}]_i\).

As described previously (4), calibration of fura 2 fluorescence in terms of \([\text{Ca}^{2+}]_i\), utilized solutions of known \(\text{Ca}^{2+}\) concentration to construct a standard curve. The \(\text{Ca}^{2+}\) concentration of the standard solutions was calculated using software (MaxChelator 6.60) that accounts for binding of \(\text{Ca}^{2+}\) to each constituent of the solution.

For analysis of fluorescence ratios recorded from cells, the equation \(\frac{[\text{Ca}^{2+}]_i}{K_d} = \beta \times \frac{[R - R_{\text{min}}]/(R_{\text{max}} - R)}{[R_{\text{max}}]/[R]}\) was used, where \(K_d\) is the dissociation constant, \(\beta\) is the ratio of fluorescence values for \(\text{Ca}^{2+}\)-free to \(\text{Ca}^{2+}\)-bound fura 2 measured at the 380 nm excitation wavelength, \(R\) is the ratio of the fluorescence intensities measured at 340 and 380 nm, and \(R_{\text{min}}\) and \(R_{\text{max}}\) are the minimum and maximum values, respectively, of the fluorescence ratio (\(R\)). The equation was fit to the standard curve (using SigmaPlot software; SPSS) and used to convert ratios (\(R\)) into \([\text{Ca}^{2+}]_i\) values (10). In situ calibration of fura 2 fluorescence by direct determination of \(R_{\text{min}}\) and \(R_{\text{max}}\) from within cells yielded similar calibrated values (not shown). Traces shown are representative of at least three similar experiments.

Separation of \[^{3}H\]arachidonic acid and \[^{3}H\]phosphatidic acid by thin layer chromatography. A7r5 cells were grown to confluence in 60-mm petri dishes and labeled for 24 h with \[^{3}H\]arachidonic acid (AA; 1 μCi in 3 ml of Dulbecco's modified Eagle's medium (DMEM) supplemented with 3.5% fetal bovine serum at 37°C). The cells were washed twice with control medium supplemented with 0.5% fatty acid-free BSA and preincubated for an additional 37.5 min in control medium supplemented with 0.1% fatty acid-free BSA in the presence or absence of 5 μM ONO-RS-082. The medium was removed and the cells were then treated with 100 μM AVP ± 5 μM ONO-RS-082 in 1.5 ml of medium for 27 min at 37°C. At the end of this incubation, the medium was removed and extracted according to the methods of Bligh and Dyer (1). The extracted lipids from the medium were dried in a SpeedVac rotary evaporator, resuspended in chloroform, and spotted on Whatman 60A-LKD thin layer chromatography (TLC) plates. The plates were developed in a solvent system of ethyl acetate, hexane, acetic acid, and water (85:35:15:90 parts, respectively). The locations of the lipids on the plate were detected using molybdenum blue reagent, and bands corresponding to locations of the unlabeled phosphatidylbutanol standard were scraped into scintillation vials and counted in an LKB-1209 liquid scintillation counter. Results were compared by one-way ANOVA and were considered statistically significant at \(P < 0.05\).

Materials. Cell-culture media were from GibCO-BRL or MediaTech. Fura 2-AM, fura 2 pentapotassium salt, and Pluronic F-127 were from Molecular Probes; EGTA (puriss. grade) was from Fluka Chemical; \[^{3}H\]AA and \[^{3}H\]palmitic acid were from American Radiolabeled Chemicals; and dipalmitoylphosphatidyl butanol was from Avanti Polar Lipids. ONO-RS-082 was from Biomol. AA from several sources was tested (Sigma, Calbiochem, Fluka, and Biomol) and was used fresh or after storage under N₂ gas. All other chemicals including AVP and PLD type VII were from Sigma.

RESULTS

AA metabolism. We previously found that the putative PLÅ2 inhibitor ONO-RS-082 blocked both AVP-stimulated \(\text{Ca}^{2+}\) spiking and AVP-stimulated release of \[^{3}H\]AA from A7r5 cells (2). Our interpretation of these findings was that \(\text{PLÅ2}\) might be involved in AVP-stimulated \(\text{Ca}^{2+}\) spiking. To determine whether the product of \(\text{PLÅ2}\), AA, is important for stimulation of \(\text{Ca}^{2+}\) spiking, fura 2-loaded A7r5 cell monolayers were treated with varying concentrations of AA (see Fig. 1). AA added to the medium at concentrations from 1 nM to 50 μM did not stimulate \(\text{Ca}^{2+}\) spiking. AA from several sources was tested and consistently failed to induce spiking, although at concentrations ≥20 μM a gradual increase in baseline \([\text{Ca}^{2+}]_i\) was observed (see Fig. L4).

It is possible that AA cleaved from membrane phospholipids exerts a local effect which is not achieved by addition of exogenous AA to the extracellular medium. AA produced via \(\text{PLÅ2}\) may be metabolized by cyclooxygenase, lipooxygenase, and cytochrome P-450 pathways to produce a variety of other signaling molecules. A number of pharmacological inhibitors of these AA metabolic pathways were tested for effects on AVP-stimulated \(\text{Ca}^{2+}\) spiking. Inhibitors of cyclooxygenase (10 μM indomethacin or 20–50 μM ibuprofen), lipooxygenase (10 μM 5,8,11-eicosatriynoic acid, 1–10 μM
AA-861, or 100 nM to 5 μM baicalein), and cytochrome P-450 (5–20 μM methoxsalen or 1 mM 1-aminobenzotriazole) were ineffective in preventing AVP-stimulated Ca^{2+}-spiking activity although some had modest stimulatory effects (data not shown). Ketoconazole (10 μM), a widely used cytochrome P-450 inhibitor, slightly inhibited AVP-induced Ca^{2+} spiking, but this concentration also inhibited [Ca^{2+}]_i changes induced by increasing extracellular K^+ concentration, which suggests a nonspecific effect on Ca^{2+} channels. These results indicate that metabolism of AA is unlikely to be required for stimulation of Ca^{2+} spiking.

Lysophospholipids. Lysophospholipids are also products of PLA_2 activity. In a series of experiments, several lysophospholipids were tested for effects on Ca^{2+} spiking. None of the compounds tested (lysophosphatidylethanolamine, lysophosphatidylcholine, lysophosphatidylserine, lysophosphatidylinositol, lysophosphatic acid, and lysophosphatidylglycerol) induced Ca^{2+} spiking at concentrations between 1 and 100 μM (not shown). In general, no effect on [Ca^{2+}]_i was observed except at the highest concentrations tested (100 μM), which in some cases (i.e., lysophosphatidylethanolamine and lysophosphatidylcholine) induced a steady increase in [Ca^{2+}]_i, that was reversed by washing away the compound.

Phospholipase inhibition by ONO-RS-082. AVP-stimulated AA release was previously assessed by labeling cells with [3H]AA and then measuring the radioactivity released into the medium after exposure to AVP (2). Similar methods were used by Ito and colleagues (11) who also found that the release of [3H]AA radioactivity was stimulated by very low concentrations of AVP (EC_{50} ≈ 50 pM). However, reports that AVP stimulates PLD in VSM (including A7r5 cells (13, 15, 23)) led us to investigate whether some of the radioactivity may be in the form of PA (the product of PLD), which may contain the labeled fatty acyl moiety. We used TLC to separate the lipid products and then determined the radioactivity that comigrates with purified AA or PA standards. The results are shown in Fig. 2 and indicate that ONO-RS-082 does not inhibit AVP-stimulated AA release (open bars), but does inhibit PA formation (closed bars). These findings suggest that ONO-RS-082 inhibits AVP-stimulated PLD rather than PLA_2 activation.

The stimulation of PA formation by AVP might be due to PLD activation or to phosphorylation of diacylglycerol. To unequivocally demonstrate activation of PLD (6), A7r5 cells were labeled with [3H]palmitic acid and then treated with AVP in the presence of 1-butanol. Under these conditions, only PLD activation will lead to an increase in the formation of [3H]-labeled phosphatidylbutanol. As shown in Fig. 3, [3H]phosphatidylbutanol was significantly increased by 500 pM AVP, and a larger increase occurred with maximal AVP stimulation (100 nM AVP). Full concentration-response curves for AVP-stimulated phosphatidylbutanol formation (see Fig. 3, inset) revealed a half-maximal stimulation at an AVP concentration of 1.09 ± 0.38 nM (n = 3).

Stimulation of Ca^{2+} spiking by PLD. Exogenous PA added to the medium at concentrations from 10 nM to...
10 μM did not stimulate Ca\(^{2+}\) spiking (not shown). However, we also examined whether cleavage of endogenous phospholipids by PLD could stimulate Ca\(^{2+}\) spiking. We tested a bacterial PLD previously shown by Jones and colleagues (13) to stimulate PA formation to a similar level as AVP in A7r5 cells. As shown in Fig. 4, PLD (2.5 U/ml) produced a Ca\(^{2+}\)-spiking response that was indistinguishable from AVP (100 pM). Mean spike amplitude was 233 ± 6 nM for 100 pM AVP and 231 ± 1.2 nM for 2.5 U/ml of PLD; spike frequencies were 5.0 and 4.6 min\(^{-1}\), respectively. Bacterial PLA\(_2\) (2.5 U/ml) had no effect on Ca\(^{2+}\) spiking (not shown).

1-Butanol prevents AVP-stimulated Ca\(^{2+}\) spiking. Butanol has been used by many groups to evaluate PLD activity because it participates in a transphosphatidylation reaction that diverts PLD away from the production of PA and toward the production of phosphatidylbutanol. 1-Butanol (0.2% vol/vol; ~22 mM) completely prevented AVP-stimulated Ca\(^{2+}\) spiking (mean spike amplitude was 199 ± 6 nM in AVP alone, and frequency was 4.8 ± 0.2 min\(^{-1}\) with no spiking.

Fig. 3. Phospholipase D (PLD) activation by AVP. PLD activity was determined by measuring \(^{3}\)Hphosphatidylbutanol (\(^{3}\)H[PBut]) formation in response to AVP in the presence of 1-butanol (see MATERIALS AND METHODS). Results from three experiments performed in duplicate are shown. *Significant difference from control; \(P < 0.001\).

Inset: a representative concentration-response curve for AVP-stimulated \(^{3}\)H[PBut] formation. [AVP], AVP concentration. Maximum stimulation based on a curve fit of the data to the equation \(y = a \times x/(b + x)\) was set at 100%.

Fig. 4. Stimulation of Ca\(^{2+}\) spiking by PLD. PLD type VII (2.5 U/ml) added to the medium stimulated Ca\(^{2+}\) spiking (bottom). This effect is indistinguishable from the effect of 100 pM AVP (top).

Fig. 5. 1-Butanol inhibits AVP- but not BaCl\(_2\)-stimulated Ca\(^{2+}\) spiking. A7r5 cells were stimulated with AVP (100 pM; top) or BaCl\(_2\) (1 mM; bottom) in the absence (left) or presence (right) of 0.2% 1-butanol. Note that estimated [Ca\(^{2+}\)], values in bottom panels have not been corrected for effects of Ba\(^{2+}\) on fura 2 fluorescence. Uncalibrated fluorescence ratios for spike peaks were 3.03 ± 0.03 for 2 mM BaCl\(_2\) alone and 3.02 ± 0.01 for BaCl\(_2\) + 0.2% 1-butanol. Mean frequency of spiking was 3.2 ± 1.7 min\(^{-1}\) for BaCl\(_2\) alone and 4.3 ± 1.3 min\(^{-1}\) for BaCl\(_2\) + 1-butanol.
observed in the presence of 0.2% of 1-butanol in four experiments; see Fig. 5, top right). This concentration of butanol did not apparently disrupt the cell membrane or adversely affect the Ca\(^{2+}\) homeostatic mechanisms because it did not affect resting [Ca\(^{2+}\)]\(_i\) levels or the increase in baseline [Ca\(^{2+}\)]\(_i\) in response to AVP (see Fig. 5, top right) or prevent stimulation of Ca\(^{2+}\) spiking by BaCl\(_2\) (see Fig. 5, bottom right). BaCl\(_2\) likely stimulates Ca\(^{2+}\) spiking by inhibiting K\(^+\) channels, a mechanism that may be an element of AVP signaling downstream of PLD activation (21). 2-Butanol, a butanol isomer that does not participate in the transphosphatidylation reaction (5), did not prevent AVP-stimulated Ca\(^{2+}\) spiking (spike frequency averaged 4.8 min\(^{-1}\) in 100 pM AVP, and 6.1 min\(^{-1}\) in AVP + 2-butanol; see Fig. 6, A and B). Phosphatidylbutanol formation was not stimulated by AVP in the presence of 2-butanol (see Fig. 6C).

Protein kinase C and PLD activation. Protein kinase C (PKC) has been implicated as either an upstream activator of PLD or a downstream effector of PLD in a variety of cell types (6, 7). In a previous study, we found that AVP-stimulated Ca\(^{2+}\) spiking is prevented by the PKC inhibitor Ro-31-8220 or by downregulation of PKC isoforms by prolonged pretreatment with 4-\(\beta\)-phorbol-12-myristate 13-acetate (PMA; see Ref. 8). AVP-stimulated PLD activity was not inhibited by PMA pretreatment or by the PKC inhibitor Ro-31-8220 (1 \(\mu\)M; see Fig. 7), which suggests that PKC activation is downstream rather than upstream in the AVP signaling cascade.

DISCUSSION

The results from the present study suggest that AVP-stimulated PA formation by PLD is both necessary and sufficient to stimulate Ca\(^{2+}\) spiking in A7r5 cells. This finding may provide an important link between PLD and vasoconstrictor activity. PLD activity has been demonstrated in many cell types including VSM, where among the known activators of PLD are the vasoconstrictor hormones AVP, ANG II, norepinephrine, and endothelin. The present findings expose a novel signal-transduction pathway in which PLD activation triggers Ca\(^{2+}\) signals that may underlie the potent vasoconstrictor effects of AVP as well as other vasoconstrictor hormones.

Although we previously suggested that PLA\(_2\) might mediate AVP-stimulated Ca\(^{2+}\) spiking (2), further investigation has led us to the conclusion that PLA\(_2\) is not the primary player in this signaling pathway. To summarize: 1) AA release was only slightly increased by 100 pM AVP and this effect was increased rather than inhibited by ONO-RS-082, whereas AVP-stimulated PA formation was completely inhibited by ONO-RS-082; 2) AA and lysophospholipids (the products of PLA\(_2\)) were ineffective in stimulating Ca\(^{2+}\) spiking; 3) agents that inhibited PLD-catalyzed PA formation (ONO-RS-082 or 1-butanol) also prevented AVP-stim-
calculated Ca\(^{2+}\) spiking; and 4) exogenous PLD, which has been shown previously to stimulate PA formation (13), stimulated Ca\(^{2+}\) spiking, whereas exogenous PLA\(_2\) did not.

The postulated role of PLD in stimulation of Ca\(^{2+}\) spiking implies a temporal sequence in which PLD activation would precede downstream signaling steps and the initiation of Ca\(^{2+}\) spiking. Unfortunately, the methods employed to detect PLD activation are not sensitive enough to detect the relatively modest increase in PLD activity at low AVP concentration at early time points. However, we do not feel that our inability to detect these rapid changes in PLD activation necessarily diminishes the potential importance of PLD in the cellular responses observed. It is increasingly apparent thatsignaling cascades operate within subcellular microdomains where minute changes in the biochemical environment can lead to profound cellular responses. Sophisticated methods have recently been developed to measure tiny subcellular [Ca\(^{2+}\)]\(_i\) changes (e.g., Ca\(^{2+}\) sparks) that are believed to play important roles in smooth muscle physiology. These Ca\(^{2+}\) sparks were impossible to resolve previously by methods that detected the larger global [Ca\(^{2+}\)]\(_i\) changes induced by larger stimuli. Similarly, more sophisticated techniques for measuring PLD activity in real time may be required to detect the earliest subcellular activity stimulated by physiological concentrations of AVP.

It remains to be determined how binding of AVP to vasopressin V\(_{1a}\) receptors activates PLD. Heterotrimeric G proteins have been implicated in PLD activation. For example, \(\beta_{3}\)-subunits and G\(_{12}\) have recently been postulated to be involved in ANG II-stimulated PLD activity in VSM (25), and G\(_{13}\) (19) has been implicated in activation of PLD in nonmuscle cells. At least two isoforms of PLD (PLD\(_1\) and PLD\(_2\)) are expressed in mammalian cells. Both of these isoforms are expressed in A7r5 cells (Ref. 9 and K. L. Byron, unpublished observation). Whereas evidence from several laboratories has suggested that PLD\(_1\) may be regulated by numerous factors including small molecular weight G proteins (e.g., ADP ribosylation factor and RhoA), PKC, and tyrosine kinases, regulation of PLD\(_2\) is still poorly characterized (for reviews, see Refs. 6 and 7).

Interestingly, the pressor responses of spontaneously hypertensive rats to AVP were reportedly inhibited by the cholesterol-lowering drug lovastatin (12), and this effect was associated with a decreased expression of small molecular weight G proteins (Ras and Rho) but not heterotrimeric G proteins (G\(_{\alpha}\), G\(_{i}\), or G\(_{q}\)). Activation of PLD by G protein-coupled receptors has been recently reported to involve a specific domain within the heptahelical receptor (14). The Asn-Pro-X-Tyr motif in the seventh transmembrane domain of rhodopsin family receptors that couple to PLD activation is postulated to form a functional complex with the small molecular weight G proteins, ARF and RhoA. This motif is also present in the seventh transmembrane domain of human and rat V\(_{1a}\) vasopressin recep-

tors (16, 22) and provides a potential link between AVP binding and PLD activation by small molecular weight G proteins.

Despite the paucity of information on the mechanisms of activation of PLD in vascular smooth muscle by G protein-coupled agonists, it is clear that a variety of vasoconstrictors activate PLD. In some instances, PLD activation by AVP has been demonstrated to occur with greater potency than the PLC-mediated formation of Ins(1,4,5)P\(_3\) (17), which suggests a qualitative as well as quantitative concentration-dependent pattern of second-messenger formation. If different signaling pathways are activated over different ranges of agonist concentration, the pathways may produce different physiological endpoints. Very high concentrations of vasoconstrictor hormones are known to lead to vascular remodeling involving hypertrophy and/or hyperplasia of smooth muscle cells. These vascular alterations have been implicated in pathological processes such as the development of atherosclerosis and hypertension. From a teleological point of view, it would be appropriate to provide for systemic regulation of smooth muscle contractility without stimulating events that may lead to vascular remodeling. Our present findings lead us to speculate that systemic concentrations of AVP in the picomolar range (below the concentrations required to induce vascular remodeling) may modulate the frequency of Ca\(^{2+}\) spiking in VSM of resistance vessels by activation of PLD and thereby regulate tissue perfusion and peripheral resistance.

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