Vasopressin-induced vasoconstriction: two concentration-dependent signaling pathways

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Henderson KK, Byron KL. Vasopressin-induced vasoconstriction: two concentration-dependent signaling pathways. J Appl Physiol 102: 1402–1409, 2007. First published January 4, 2007; doi:10.1152/japplphysiol.00825.2006.—Current scientific literature generally attributes the vasoconstrictor effects of [Arg8]vasopressin (AVP) to the activation of phospholipase C (PLC) and consequent release of Ca2+ from the sarcoplasmic reticulum. However, half-maximal activation of PLC requires nanomolar concentrations of AVP, whereas vasoconstriction occurs when circulating concentrations of AVP are orders of magnitude lower. Using cultured vascular smooth muscle cells, we previously identified a novel Ca2+ signaling pathway activated by 10–100 pM AVP. This pathway is distinguished from the PLC pathway by its dependence on protein kinase C (PKC) and L-type voltage-sensitive Ca2+ channels (VSCC). In the present study, we used isolated, pressurized rat mesenteric arteries to examine the contributions of these different Ca2+ signaling mechanisms to AVP-induced vasoconstriction. AVP (10^−14–10^−6 M) induced a concentration-dependent constriction of arteries that was reversible with a V1a vasopressin receptor antagonist. Half-maximal vasoconstriction at 30 pM AVP was prevented by blockade of VSOC with verapamil (10 μM) or by PKC inhibition with calphostin-C (250 nM) or Ro-31-8220 (1 μM). In contrast, acute vasoconstriction induced by 10 nM AVP (maximal) was insensitive to blockade of VSOC or PKC inhibition. However, after 30 min, the remaining vasoconstriction induced by 10 nM AVP was partially dependent on PKC activation and almost fully dependent on VSOC. These results suggest that different Ca2+ signaling mechanisms contribute to AVP-induced vasoconstriction over different ranges of AVP concentration. Vasoconstrictor actions of AVP, at concentrations of AVP found within the systemic circulation, utilize a Ca2+ signaling pathway that is dependent on PKC activation and can be inhibited by Ca2+ channel blockers.

pressurized artery; antidiuretic hormone; protein kinase C; vascular smooth muscle; signal transduction

[ARG8]VASOPRESSIN (AVP) is a nine-amino acid peptide hormone that is released from the posterior pituitary gland into the systemic circulation in response to an increase in plasma osmolality and/or a reduction in blood pressure. AVP exerts an antidiuretic effect at the kidneys by binding to V2 receptors. Additionally, AVP binds to V1a receptors on vascular smooth muscle cells to induce vasoconstriction. Skin, muscle, and splanchic arterial beds are highly sensitive to the vasoconstrictor actions of AVP (30), an effect that may be important for shunting blood to more vital organs. Rat mesenteric arteries were found to be particularly sensitive to vasopressin and to contract in response to concentrations of AVP as low as 1 pM (2). In humans, AVP is one of the most potent vasoconstrictors, and hence circulating concentrations of AVP in healthy individuals with normal blood pressure and hydration are generally <3 pM (32). With dehydration, circulating concentrations of AVP may exceed 10 pM and during hypovolemic shock increase up to 500 pM (4, 14, 37). Importantly, under physiological conditions, circulating concentrations of AVP rarely exceed 100 pM (32, 37).

Clinical use and awareness of AVP and its receptor antagonists are increasing within the medical community. AVP concentrations are chronically and significantly elevated in patients with congestive heart failure (18, 19), such that it has been deemed an appropriate marker for the presence and severity of this disease (33). Because AVP regulates vascular tone and free-water absorption via V1a and V2 receptors, respectively, these receptors have become pharmacological targets for the treatment of congestive heart failure, and receptor antagonists are currently being tested in clinical trials (17, 19, 45). V1a antagonists may also be effective for the treatment of vasospasm in subarachnoid hemorrhage. In a rat model of subarachnoid hemorrhage, both plasma AVP concentration and sensitivity to AVP were increased (36, 44). This effect was accompanied by basilar artery vasospasm, which was attenuated by a V1a vasopressin receptor antagonist. Vasopressin is also an effective clinical therapy for patients with vasodilatory shock, which is refractory to catecholamine administration (12, 31).

It is generally accepted that the physiological and pathophysiological effects of AVP on the vasculature involve phospholipase C (PLC) activation (21, 34, 39, 43, 46). Numerous studies have demonstrated that AVP binding to V1a vasopressin receptors leads to PLC-mediated production of inositol 1,4,5-trisphosphate (IP3) and, consequently, induces Ca2+ release from the sarcoplasmic reticulum (SR) (34, 43). This signaling pathway is robustly activated by AVP in vascular smooth muscle cells, including the A7r5 cell line, which is derived from embryonic rat thoracic aorta (8, 9, 13, 25). However, half-maximal activation of PLC requires concentrations of AVP that are 100- to 1,000-fold higher than the picomolar concentrations of AVP measured in the systemic circulation (13). Because picomolar concentrations of AVP have been shown to induce vasoconstriction in vivo (2), we raised the question as to whether there are alternative Ca2+ signaling mechanisms that are more sensitive to AVP that may account for the vasoconstrictor effects of circulating AVP.

Using cultured A7r5 vascular smooth muscle cells, our laboratory previously identified an alternative Ca2+ signaling pathway that is activated by picomolar concentrations of AVP (8–10, 15, 28). Specifically, these studies demonstrated a steep concentration-dependent increase in Ca2+ transients (Ca2+ spikes), at concentrations of AVP within the physiological

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concentration range of 10–500 pM (9). Protein kinase C (PKC) activation and Ca$^{2+}$ entry through voltage-sensitive L-type Ca$^{2+}$ channels (VSCC) are required for picomolar AVP-induced stimulation of Ca$^{2+}$ spiking in A7r5 cells (9, 15). Concentrations of AVP >100 pM activate Ca$^{2+}$ signals associated with PLC activation, independent of L-type VSCC (8).

It is not known whether the novel Ca$^{2+}$-signaling pathway for low AVP concentrations elucidated in immortalized A7r5 cells can account for the vasoconstrictor effects of circulating AVP. We hypothesized that picomolar concentrations of AVP would induce vasoconstriction through the same PKC- and L-type VSCC-dependent pathway. This hypothesis was tested in the present study, and the results suggest the presence of two concentration-dependent Ca$^{2+}$ signaling pathways for AVP-induced vasoconstriction.

MATERIALS AND METHODS

Adult male Sprague-Dawley rats (N = 43, 300–350 g) were obtained from Harlan (Indianapolis, IN). Animal protocols were approved by the Loyola University Chicago Institutional Animal Care and Use Committee. Rats were anesthetized by inhalation of 4% isoflurane, a midline abdominal incision was made, and the abdominal cavity was filled with ice-cold physiological saline solution (PSS) containing the following (in mM): 145 NaCl, 4.7 KCl, 1.2 NaH$_2$PO$_4$, 1.2 MgSO$_4$, 2 CaCl$_2$, 2 H$_2$O, 2 pyruvic acid, 0.02 EDTA dihydrate, 3 MOPS, and 5 d-glucose. PSS + albumin contained 1% fatty acid free bovine serum albumin. The small intestinal mesentery was carefully removed and placed into ice-cold PSS + albumin (pH = 7.4 at 37°C).

Arterial isolation and cannulation techniques by Davis et al. (11) were used. Briefly, in a cooled dissecting chamber (5°C), fourth-order mesenteric large resistance arteries (~250–300 μm in outer diameter, branching immediately adjacent to the small intestine) were isolated and cleaned of adventitia. Arteries were transferred to a 10-ml bath containing PSS. Osmolarity of PSS solutions with albumin was adjusted to ~298 mosM with water to match the osmolarity of the PSS solution without albumin; this resulted in a <1% change in solute concentrations. Vessels were cannulated with glass micropipettes filled with PSS + albumin (pH = 7.4 at 37°C), secured with nylon suture, and gradually pressurized to 80 mmHg. This pressure is based on in vivo pressure measurements of the mesenteric circulation (16).

Vessel pressure and patency were verified by in-line pressure transducers on either side of the vessel. The artery was gently washed by superfusion with 60-ml cold PSS (pH = 7.4 at 37°C), slowly warmed to 37°C, and allowed to equilibrate for 1 h before experimental protocols. External diameter of the artery, temperature, and pressure were electronically measured in real time using a pressure myograph system (DMT-USA, Atlanta, GA). Maximal vessel diameter at 80 mmHg was measured in PSS with zero Ca$^{2+}$ and 100 μM sodium nitroprusside or a combination of 10 μM cyclopiazonic acid and 2.5 mM caffeine to deplete the SR Ca$^{2+}$ stores (similar results were obtained using either method). Results are presented as outer vessel diameter, in micrometers. Summary data, representing experimentally induced changes in vessel diameter under various conditions, were normalized and are shown as a percentage of maximal outer diameter.

Vasopressin was added to the arterial bath to attain incremental log and half-log increases in AVP concentration ([AVP]) (10$^{-14}$–10$^{-6}$M). Treatments were applied at 5-min intervals, or after vasoconstrictor responses had reached a plateau or were stable for 1 min. PSS (100 μl) was initially added to the bath as a vehicle control. The lowest concentration of AVP resulting in significant vasoconstriction and the lowest concentration resulting in maximal vasoconstriction were used to examine AVP signal transduction pathways.

Calphostin-C and Ro-31-8220 were obtained from BIOMOL Research Laboratories (Plymouth Meeting, PA). Other chemicals and drugs were obtained from Sigma-Aldrich (St. Louis, MO). Ro-31-8220 and verapamil were protected from light to prevent inactivation, whereas calphostin-C experiments were conducted in room light to ensure its activation.

Data are presented as means ± SE and were analyzed with SigmaStat (SPSS Scientific) and Prism software. T-tests and one-way repeated-measures ANOVA, followed by a Holm-Sidak post hoc test, were used for comparing responses. EC$_{50}$ values were calculated with a nonlinear regression curve fit based on sigmoid log dose-response relationships. Differences were considered significant at P < 0.05.

RESULTS

**AVP concentration-response relationship.** Vasoconstrictor responses to increasing concentrations of AVP (10$^{-14}$–10$^{-6}$) were tested in isolated, pressurized mesenteric arteries [Fig. 1, A (representative tracing) and B (summary)]. The effective concentration of AVP resulting in 50% maximal vasoconstriction was ~30 pm (log EC$_{50}$ = -10.73 ± 0.4; N = 6). One-way repeated-measures ANOVA revealed that 30 pm AVP was the lowest concentration of AVP, resulting in significant vasoconstriction. AVP (10 nM) was the lowest concentration of AVP resulting in maximal vasoconstriction (complete occlusion of the vessel, Fig. 2A).

**Acute vasoconstriction induced by 30 pM and 10 nM AVP.** It is well established that repeated exposures of arteries to high concentrations of AVP leads to tachyphylaxis (20), whereas this effect is less apparent or absent at lower concentrations of AVP (100–500 pM; Ref. 22). Our initial studies determined that responses to 30 pM and 10 nM AVP would not be altered by prior exposure to 30 pM AVP (data not shown). With naive arteries, acute addition of 30 pM AVP to the vessel bath induced significant vasoconstriction [from 297 ± 20 to 232 ± 21 μm outer diameter, n = 7, P < 0.001; Fig. 2A and Fig. 6 (summary)]. This response was reversed by superfusion exchange of the arterial bath with PSS. Subsequent addition of 10 nM AVP induced complete occlusion of the vessel (mean outer diameter 148 ± 9 μm, n = 7, Figs. 2A and 6), which was also reversed with superfusion exchange. At the end of these experiments, membrane depolarization with 60 mM KCl also produced significant vasoconstriction (P < 0.001) in four of four vessels tested (Fig. 2A), indicating the presence of functional voltage-sensitive Ca$^{2+}$ channels and vessel viability. Vessel diameters before treatments with 30 pM and 10 nM AVP, or KCl, were not significantly different. Additionally, maximal diameters measured at the end of these experiments were not significantly different from beginning vessel diameters (data not shown).

**V$_{1a}$ vasopressin receptors.** To verify that vasoconstriction induced by AVP was mediated by V$_{1a}$ vasopressin receptors, the selective V$_{1a}$ vasopressin receptor antagonist [β-mercapto-β-cyclopentamethylenepropionyl], O-me-Tyr$^2$. Arg$^8$]vasopressin was added to the vessel bath after constrictor responses to AVP had stabilized. In these experiments, the constrictor responses to 30 pm and 10 nM AVP were quickly and completely reversed by adding a 100-fold excess of the V$_{1a}$ vasopressin receptor antagonist (3 nM and 1 μM, respectively, Figs. 2B and 6).

**Role of voltage-dependent L-type Ca$^{2+}$ channels in AVP-induced vasoconstriction.** To test the hypothesis that 30 pM AVP-induced vasoconstriction is mediated by Ca$^{2+}$ entry via VSCC, 10 μM verapamil (a selective L-type Ca$^{2+}$ channel blocker) was added to the vessel bath 5 min before AVP.
administration. In the presence of verapamil, 30 pM AVP did not induce significant vasoconstriction (mean outer diameters of 284 ± 16 and 281 ± 17 μm at baseline and following 30 pM AVP, respectively, n = 5, Figs. 3 and 6). In contrast, the acute vasoconstriction induced by 10 nM AVP was not different in the presence or absence of verapamil [full vessel occlusion; 139 ± 10 μm mean outer diameter (n = 5) in the presence of 10 nM AVP plus 10 μM verapamil, Figs. 3 and 6]. The effectiveness of verapamil as an inhibitor of VSCC was verified by exposure of verapamil-treated vessels to 60 mM KCl. In four of four experiments, this protocol resulted in no significant vasoconstriction (276 ± 19 to 276 ± 18 μm before and after addition of KCl, respectively, n = 4). Collectively, these data suggest that 1) 10 μM verapamil effectively inhibits VSCC; 2) vasoconstrictor responses to 30 pM AVP are fully dependent on Ca^{2+} entry via VSCC; and 3) the acute constric-

tor response to 10 nM AVP occurs independently of VSCC.

Role of PKC in AVP-induced vasoconstriction. To test the hypothesis that 30 pM AVP-induced vasoconstriction is also PKC dependent, two selective PKC inhibitors, calphostin-C (250 nM) and Ro-31-8220 (1 μM), were used. In previous studies, these concentrations completely inhibited AVP-stimulated Ca^{2+} spiking in A7r5 cells (15). No significant vasoconstriction was observed when 30 pM AVP was added to the vessel bath in the presence of calphostin-C or Ro-31-8220 (Figs. 4 and 6). On the other hand, acute vasoconstriction induced by 10 nM AVP was not inhibited by calphostin-C or Ro-31-8220 (Figs. 4 and 6). The effectiveness of PKC inhibition by calphostin-C or Ro-31– 8220 was verified by the addition of a PKC activator, 4β-phorbol 12-myristate 13-acetate (PMA, 100 nM), into the vessel bath at the end of the experiments. For reference, acute administration of 100 nM PMA in naive arteries induced significant vasoconstriction from 290 ± 21 to 186 ± 22 μm outside diameter (n = 4). Ro-31-8220 completely blocked PMA-induced vasoconstriction (with Ro-31-8220 in the bath, vessel diameters were 332 ± 21 and 331 ± 21 μm in the absence or presence of PMA, respectively, n = 5), and calphostin-C blocked ~60% of the PMA-induced vasoconstriction (PMA constricted vessels from 322 ± 8 to 281 ± 18 μm outside diameter, in the presence of calphostin-C, n = 4). The combined results suggest that 30 pM AVP-induced vasoconstriction is PKC dependent, whereas acute vasoconstriction induced by 10 nM AVP does not require PKC activation.

In a separate series of experiments, we investigated whether PKC activation could lead to vasoconstriction, and, if so, whether PKC-dependent vasoconstriction is induced by Ca^{2+} entry via VSCC. For these studies, PMA (100 nM) was added
to the vessel bath; this induced significant vasoconstriction within 10 min ($P < 0.05$; Fig. 5). Once the constrictor response had stabilized, verapamil (10 μM) was added to the bath. Verapamil addition rapidly reversed vasoconstriction and restored the vessel to its original diameter. These data suggest that PKC activation leads to vasoconstriction, which is dependent on Ca$^{2+}$ entry via VSCC.

**Acute vs. prolonged 10 nM AVP-induced vasoconstriction.** In cultured vascular smooth muscle cells, nanomolar concentrations of AVP induce Ca$^{2+}$ release from intracellular stores by a process that is insensitive to Ca$^{2+}$ channel blockers or PKC inhibition (6, 8). This effect probably accounts for our observation that the acute vasoconstrictor response to 10 nM AVP was not sensitive to verapamil or PKC inhibition. However, prolonged Ca$^{2+}$ release from the SR is limited by its storage capacity, Ca$^{2+}$ reuptake, and Ca$^{2+}$ efflux. Therefore, in a separate series of experiments, we investigated whether Ca$^{2+}$ release continues to contribute to sustained 10 nM AVP-induced vasoconstriction. In these studies, 10 nM AVP induced maximal vasoconstriction (occlusion of the artery) and after 30 min retained ~70% of the initial vasoconstrictor response (Fig. 7). Interestingly, the remaining vasoconstriction was reversed by verapamil (10 μM), relaxing arteries to ~94% of their maximal diameter. When arteries were preincubated with verapamil, 10 nM AVP induced complete occlusion of the artery, but, after 30 min, the arteries had relaxed to ~91% of their maximal diameter (Fig. 7). Combined, these data suggest that, whereas acute constriction is independent of VSCC, the vast majority of sustained vasoconstriction induced by 10 nM AVP (at 30 min) is dependent on these channels.

The results with verapamil suggest a transition from Ca$^{2+}$ release to Ca$^{2+}$ entry during continuous exposure to 10 nM AVP. To determine whether this transition from Ca$^{2+}$ release to Ca$^{2+}$ entry involves PKC, we preincubated arteries with...
arteries (PKC activation, Ca\textsuperscript{2+} entry, and Ca\textsuperscript{2+} release) are similar to the concentration-dependent signaling pathways identified in A7r5 cells.

AVP concentration-dependent transition between Ca\textsuperscript{2+} entry and Ca\textsuperscript{2+} release. Our laboratory’s previous studies (7, 10) demonstrated that voltage-gated K\textsuperscript{+} channels are regulated in a PKC-dependent manner at low AVP concentrations in A7r5 cells, such that PKC-dependent inhibition of outward K\textsuperscript{+} currents leads to membrane depolarization and Ca\textsuperscript{2+} entry via VSCC. If these mechanisms operate in intact arteries, they would account for both the PKC dependence and verapamil sensitivity of vasoconstrictor responses at 30 pM AVP observed in the present study. In A7r5 cells, nanomolar AVP concentrations activate PLC, which leads to IP3 production and the release of intracellular Ca\textsuperscript{2+}. A similar Ca\textsuperscript{2+} response in intact arteries probably accounts for the acute vasoconstrictor effects of 10 nM AVP that were insensitive to verapamil or PKC inhibition.

Pioneering work by van Breemen and others in the early 1980s demonstrated that high agonist concentrations would elicit a biphasic constrictor response generated by Ca\textsuperscript{2+} release followed by Ca\textsuperscript{2+} entry (26). Indeed, there is now widespread agreement and abundant evidence that early (transient) and late (sustained) phases of constriction at high concentrations of a variety of G protein-coupled vasoconstrictor agonists can be attributed (in most cases) to IP3-mediated Ca\textsuperscript{2+} release and Ca\textsuperscript{2+} entry via L-type channels, respectively. However, neither the concentration dependence for these responses nor the signal transduction mechanisms mediating these responses have been examined systematically, with particularly little information available for constrictor responses at physiological concentrations of AVP. Our findings provide new evidence that there is a concentration- and time-dependent divergence of signal transduction mechanisms that mediate the recruitment of

calphostin-C (250 nM). Again, 10 nM AVP induced complete occlusion, but after 30 min arteries were significantly more relaxed in the presence of calphostin-C vs. control (P = 0.01; Fig. 7), and the remaining vasoconstriction was abolished with verapamil. These data suggest that the sustained vasoconstrictor effects of 10 nM AVP (at 30 min) are largely dependent on L-type VSCC and partially dependent on PKC.

**DISCUSSION**

Our findings demonstrate that, in mesenteric arteries maintained under physiological ionic conditions, temperature, and intravascular pressure, AVP concentration-dependent vasoconstriction is mediated by two distinct Ca\textsuperscript{2+} signaling mechanisms. Concentrations of AVP found within the systemic circulation induce vasoconstriction, which is fully dependent on PKC activation and VSCC. In contrast, a concentration of 10 nM AVP acutely induces maximal vasoconstriction independently of these mechanisms, but sustained vasoconstriction reverts to a mechanism that requires both PKC and VSCC for its full effect.

**A7r5 cells vs. mesenteric arteries.** A7r5 vascular smooth muscle cells were derived from embryonic rat aorta (25) and have been used for several decades as a model for vascular smooth muscle cell signal transduction and electrophysiology studies. Our studies in A7r5 cells have previously demonstrated that picomolar concentrations of AVP stimulate Ca\textsuperscript{2+} spiking by a pathway that is dependent on PKC (10, 15) and Ca\textsuperscript{2+} entry via L-type VSCC (9). In the same cells, nanomolar to micromolar concentrations of AVP induce release of intracellular Ca\textsuperscript{2+} stores independently of PKC or L-type Ca\textsuperscript{2+} channels (6, 8, 15). The present study suggests that the signaling pathways activated by AVP in freshly isolated, pressurized, mesenteric large resistance arteries (PKC activation, Ca\textsuperscript{2+} entry, and Ca\textsuperscript{2+} release) are similar to the concentration-dependent signaling pathways identified in A7r5 cells.

![Fig. 4. Inhibition of protein kinase C (PKC) abolishes 30 pM AVP-induced vasoconstriction, but responses to 10 nM AVP are not inhibited. Pressurized arteries were preincubated with the PKC inhibitor Ro-31-8220 (1 μM) for 30 min and then throughout the experiment. Application of 30 pM AVP did not generate vasoconstriction, whereas 10 nM AVP induced significant vasoconstriction. Summarized results are shown in Fig 6.](https://www.jap.org/)

![Fig. 5. PKC activation leads to vasoconstriction via activation of L-type Ca\textsuperscript{2+} channels. Addition of the PKC activator, 4α-phorbol 12-myristate 13-acetate (PMA, 100 nM), to the vessel bath induced significant vasoconstriction (*P < 0.05). Once constriction had reached a plateau with PMA, verapamil (10 μM) was added to the arterial bath and significantly reversed PMA-induced vasoconstriction (P < 0.05). Results are means ± SE for 4 experiments.](https://www.jap.org/)
these different sources of Ca\(^{2+}\) for activation of mesenteric artery constriction in response to vasopressin.

**PLC, diacylglycerol, and PKC.** Agonist-stimulated PLC activation leads to diacylglycerol (DAG) production. DAG is a well-known activator of PKC, but it can also directly activate nonselective cation channels (24, 42). The latter effect would provide an additional depolarizing stimulus for the activation of VSCC (46). This might account for the sustained vasoconstrictor responses to 10 nM AVP that were sensitive to verapamil, but insensitive to PKC inhibition.

According to our hypothetical scheme shown in Fig. 8, the effects of PKC would depend on the concentration of AVP. At low AVP concentrations, PKC-dependent regulation of voltage-activated K\(^+\) (K\(_v\)) channels would stimulate Ca\(^{2+}\) influx via L-type VSCC (the major vasoconstrictor stimulus at low AVP concentrations). At higher AVP concentrations, PLC activation results in production of IP\(_3\) and DAG and further activation of PKC. PKC activation by phorbol esters or high agonist concentrations has been implicated in a number of cellular processes that either positively [inhibition of Ca\(^{2+}\) sparks (5); increased sensitivity of the contractile proteins (38)] or negatively [decreased nonselective cation currents (1); increased Ca\(^{2+}\) extrusion from the cytosol (6)] influence vascular smooth muscle contraction. PKC inhibition would prevent these effects as well as the contribution of K\(_v\) channel regulation, but it would not be expected to influence IP\(_3\)-mediated Ca\(^{2+}\) release or DAG-dependent activation of nonselective cation channels. In the response to 10 nM AVP, the activation of nonselective cation channels and resulting membrane depolarization would account for the majority of the sustained vasoconstriction that was insensitive to PKC inhibition but was sensitive to blockade of VSCC. Further studies will be required.

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**Fig. 6.** Concentration-dependent switch in signal transduction for AVP-induced vasoconstriction. Summarized results are shown, normalized to a percentage of maximal outer diameter. 30 pM AVP induced significant vasoconstriction, which was completely reversed with a V\(_{1a}\) vasopressin receptor antagonist, and significantly inhibited by the voltage-sensitive L-type Ca\(^{2+}\) channel blocker, verapamil (Verap, 10 \(\mu\)M), as well as the PKC inhibitors, calphostin-C (Cal-C, 250 nM) and Ro-31-8220 (Ro, 1 \(\mu\)M) (*\(P < 0.05\)). 10 nM AVP induced significant vasoconstriction, which was completely reversed by the V\(_{1a}\) vasopressin receptor antagonist (*\(P < 0.05\)), but was not affected by verapamil or PKC inhibitors.

**Fig. 7.** Acute vasoconstriction induced by 10 nM AVP was similar in the presence or absence of verapamil (10 \(\mu\)M) or calphostin-C (250 nM). *All arteries significantly relaxed after 30 min of 10 nM AVP exposure (\(P < 0.05\)). Arteries preincubated with verapamil or calphostin-C relaxed significantly more than control arteries at 30 min (\(\delta P < 0.001\) and \(P = 0.011\), respectively). †Acute addition of verapamil 30 min after 10 nM AVP induced significant relaxation in the presence or absence of calphostin-C (*\(P < 0.05\)). There was also slightly, but significantly, more relaxation in verapamil-treated arteries preexposed to calphostin-C vs. control arteries (#\(P = 0.014\)).
to fully elucidate whether these signal transduction pathways are activated by low and high AVP concentrations.

One receptor, two mechanisms, different concentration-dependencies. According to our hypothesis, binding of vasopressin to a single receptor subtype (V1a receptors) can result in activation of two signaling pathways. Our results, which suggest that one pathway is turned on at much lower concentrations of AVP than the other pathway, may be explained according to well-established receptor pharmacology theory. We postulate that, in the case of the low [AVP] response, elements of the signaling pathway downstream of the receptor limit the response [a “spare receptor” response (48)], whereas the high [AVP] response is proportional to the fraction of occupied receptors.

If the low [AVP] effect depends on PKC-mediated phosphorylation of K+ channels, we may speculate that the K+ channels are fully phosphorylated when only a small fraction (~5%) of the V1a vasopressin receptors are activated. Such a “spare receptor” response is expected to result in an apparent leftward shift and a steeper concentration-response relationship compared with the ligand binding curve (48). Despite the limitation of K+ channel phosphorylation, the resulting membrane depolarization is a very potent stimulus for increasing Ca2+ entry via L-type VSCC, resulting in significant vasoconstriction at a concentration of only 30 pM AVP.

This “spare receptor” scenario would dictate that activation of a larger fraction of receptors by increasing [AVP] into the nanomolar range will have no further effect on the PKC/Kv/L-type Ca2+ channel pathway, but is expected to increase PLC activity in proportion to the fraction of occupied receptors, until the receptors are all occupied. This is expected to produce increasing IP3-induced Ca2+ release responses with an EC50 approximately equal to the dissociation constant for ligand binding (~2 nM). Although more complicated models could be envisaged to account for the different concentration dependencies of the two AVP-activated signaling pathways, the spare receptor hypothesis may be the simplest way to explain the more sensitive activation of constriction via PKC/L-type Ca2+ channels.

Local AVP release and paracrine/autocrine signaling. To what extent are the effects of high AVP concentrations evoked physiologically, if plasma AVP concentrations rarely exceed 100 pM? Extrahypothalamic production of AVP has been demonstrated in the spinal cord (23), ovary and testis (35, 47), adrenal gland (3), and more recently in vascular smooth muscle cells (27, 40, 41). The vascular production of AVP suggests that vasoconstriction may occur in response to locally produced AVP. One might imagine a scenario in which relatively high concentrations of AVP are attained in the interstitial spaces and act in a paracrine/autocrine manner on the vascular smooth muscle cells of the artery wall. According to this scenario, the elevated local concentrations could activate PLC and induce focal vasoconstriction or vascular spasm. More importantly, chronic activation of PLC and elevated intracellular Ca2+ concentrations could also activate early response genes such as c-Fos and c-Jun (29), leading to localized vascular remodeling.

Summary. These studies demonstrate that the picomolar concentrations of AVP found in the systemic circulation induce significant V1a receptor-mediated vasoconstriction in freshly isolated, pressurized mesenteric arteries. This response is fully dependent on PKC activation and Ca2+ entry via VSCC. However, our results also suggest that the sources of Ca2+ that contribute to AVP-induced vasoconstriction switch from a purely L-type Ca2+ channel-dependent pathway at circulating concentrations of AVP to the better characterized PLC-mediated Ca2+ signaling pathways at nanomolar AVP levels. The former may account for the endocrine actions of AVP that adjust moment-to-moment blood flow and pressure, whereas the latter may occur at sites of local vascular AVP production. These concentration-dependent differences in Ca2+ signaling may provide important clues to understand the etiology and to develop more effective therapies to treat cardiovascular diseases in which vasopressin plays an important role.

GRANTS

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