Arginine vasopressin inhibits Kir6.1/SUR2B channel and constricts the mesenteric artery via V1a receptor and protein kinase C

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Shi W, Cui N, Shi Y, Zhang X, Yang Y, Jiang C. Arginine vasopressin inhibits Kir6.1/SUR2B channel and constricts the mesenteric artery via V1a receptor and protein kinase C. Am J Physiol Regul Integr Comp Physiol 293: R191–R199, 2007; doi:10.1152/ajpregu.00047.2007.—Kir6.1/SUR2B channel is the major isoform of KATP channels in the vascular smooth muscle. Genetic disruption of either subunit leads to dysregulation of vascular tone and regional blood flows. To test the hypothesis that the Kir6.1/SUR2B channel is a target molecule of arginine vasopressin (AVP), we performed studies on the cloned Kir6.1/SUR2B channel and cell-endogenous KATP channel in rat mesenteric arteries. The Kir6.1/SUR2B channel was expressed together with V1a receptor in the HEK-293 cell line. Whole cell currents of the transfected HEK cells were activated by KATP channel opener pinacidil and inhibited by KATP channel inhibitor glibenclamide. AVP produced a concentration-dependent inhibition of the pinacidil-activated currents with IC50 2.0 nM. The current inhibition was mediated by a suppression of the open-state probability without effect on single-channel conductance. Indeed, previous studies have suggested that the modulation of the vascular KATP channels by AVP is still controversial (6, 38). There is evidence that KATP channels in cardiac myocytes and the insulinoma cell line are also inhibited by AVP, suggesting that Kir6.1/SUR2B channels are important regulators of membrane potentials. Indeed, previous studies have suggested that the ATP-sensitive K+ (KATP) channels are inhibited by AVP leading to depolarization of vascular smooth muscle cells (38).

As the major vascular isoform, the Kir6.1/SUR2B channel plays an important role in vascular tone regulation. Kir6.1 knockout mice exhibited a high rate of sudden death associated with spontaneous electrocardiographic ST elevation followed by atrioventricular block, which resembles Prinzmetal angina in humans (23). Genetic disruption of the abcc9 (SUR2) gene leads to coronary artery vasospasm and raises resting blood pressures (5). The spontaneous coronary vasospasm persists in the abcc9: knockout mice with restored expression of KATP channels in vascular smooth muscles, suggesting that a process extrinsic to the coronary arterial smooth muscle may be involved (17). Vascular KATP channels are targeted by several vasoactive hormones and neurotransmitters (3). However, the modulation of the vascular KATP channels by AVP is still controversial (6, 38). There is evidence that KATP channels in cardiac myocytes and the insulinoma cell line are also inhibited by AVP, suggesting that Kir6.1/SUR1 and Kir6.2/SUR2A channels are targeted (21, 36). Since functional vascular KATP channels are mainly made of Kir6.1 with SUR2B subunits, the understanding of KATP channel contribution to vascular tones relies on the demonstration of the precise signal network between neurotransmitters/hormones and KATP channels. To test the hypothesis that Kir6.1/SUR2B channel is one of the effectors of AVP, we performed these studies. Our results indicated that the Kir6.1/SUR2B channel was inhibited by AVP through the V1a receptor, and the channel inhibition relied on intracellular signal systems involving PKC.

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MATERIALS AND METHODS

Cell culture. Rat Kir6.1 (GenBank accession no. D42145) and mouse SUR2B (GenBank accession no. D86038) were cloned in a eukaryotic expression vector, pcNDA3.1, and used for mammalian cell expression. Human AVP receptor 1A with NH2-terminal 3XHA tag (AVPR1A, GenBank accession no. AY322550) in pcNDA3.1 was purchased from http://www.cDNA.org (Rolla, MO). Wild-type V1a receptor was prepared by removing 3XHA-tag with PCR and was cloned into pcNDA3.1.

Human embryonic kidney cells (HEK-293, CRL-1573, batch no. 2187595; American Type Culture Collection, Manassas, VA) were chosen to express the KATP channels. The HEK-293 cells were cultured as monolayers in the DMEM-F12 medium with 10% fetal bovine serum and penicillin/streptomycin. Maintained at 37°C with 5% CO2 in atmospheric air, the cells were routinely split twice a week. The cells that had clear smooth muscle morphology and did not show evident swelling or shrinkage were used for patch studies.

The HEK-293 cells were transfected using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) in which 0.7 µg Kir6.1, 2.1 µg SUR2B, and 1.8 µg V1a receptor were added to a 35-mm petri dish. To facilitate the identification of positively transfected cells, 0.5 µg green fluorescent protein (GFP) cDNA (pEGFP-N2; Clontech, Palo Alto, CA) was added to the cDNA mixture. Cells were dissociated from the monolayer using 0.25% trypsin ~24 h posttransfection. A few drops of the cell suspension were added on to 5 × 5-mm coverslips in a 35-mm petri dish. The cells were then incubated at 37°C for 24–48 h before experiments.

Acute dissociation of vascular smooth myocytes. The surgical procedure for dissection of mesenteric arteries has been described previously (39). All animal experiments complied with the Institutional Animal Care and Use Committee approval of the Georgia State University. Sprague-Dawley rats (250–350 g) were anesthetized by inhalation of saturated halothane vapor followed by cervical dislocation. Mesenteric arteries were dissected free, cut into small segments (1–2 mm), and placed in 5-ml solution containing (in mM): 140 NaCl, 5.4 KCl, 1 MgCl2, 0.1 CaCl2, 10 HEPES, and 10 D-glucose at room temperature for 10 min. The tissues were then placed in 1-ml solution containing 20 units of papain ( Worthington) and 1.25 mg DTT. After digestion for 30 min at 35°C, the tissue was washed once and then transferred to 1-ml solution containing 440 units of collagenase (CLS II; Worthington) and 1.25 mg trypsin inhibitor (Sigma) for 15 min. After being thoroughly washed, the tissue was moved to 1-ml solution containing 20% fetal bovine serum and triturated with a fire-polished Pasteur pipette to yield single smooth muscle cells. The cells were stored on ice and used within 8 h. A drop of cells was put in a 35-mm tissue culture dish and the cells were allowed to attach to the surface. The cells that had clear smooth muscle morphology and did not show evident swelling or shrinkage were used for patch studies.

Patch-clamp recordings. Patch-clamp experiments were performed at room temperature as described previously (39). In brief, fire-polished patch pipettes with resistance of 4 to 6 MΩ were made with 1.2-mm borosilicate glass capillaries. Whole cell recording was performed in single-cell voltage clamp. Current records were low-pass filtered (2 kHz, Bessel 4-pole filter, ~3 dB), digitized (20 kHz, 16-bit resolution), and stored on a computer hard drive for later analysis. The filtered (2 kHz, Bessel 4-pole filter, ~3 dB) currents from 100 to 1,000 mV were recorded as current levels. The reversal potential of K+ was ~0 mV. The cell was kept at current levels corresponding to j = 0, 1, 2, . . . N channels open, based on all obvious openings during the entire period of recording. Po was then obtained as

\[ P_o = \left( \sum_{j=1}^{N} j \right) / T \cdot N, \]

where N is the number of channels active in the patch, and T is the duration of recordings. Po values were calculated from one to three stretches of data of 20 s each acquired with Clampex 8 (Axon Instruments). In this study, we used Np, instead of Po, to express overall channel activity in which the number of openings was not counted.

Isolated mesenteric arterial ring. Mesenteric arteries were obtained as mentioned above, and two to four animals were used in the study in each group. The endothelium of the rat mesenteric arteries was kept intact because the contractile responses to AVP in the rat mesenteric artery are not critically dependent on the endothelium (32). The endothelium-free rings were prepared by gently rubbing with a sanded polyethylene tube and confirmed with vasodilation response to acetylcholine. The isolated mesenteric arteries were transferred to ice-cold Krebs solution containing NaCl 118.0, NaHCO3 25.0, KCl 3.6, MgSO4 1.2, KH2PO4 1.2, glucose 11.0, CaCl2 2.5 in mM (42). A ring segment 2 mm in length was mounted on a force electricity transducer (model FT-302; iWorx/CBSciences, Dover, NH) in a tissue bath with 5 ml Krebs solution. With a 0.8 g preload added, the rings were allowed to equilibrate in the tissue bath for 30 min, and the tension then was reduced to ~0.6 g. The tissue bath was filled with Krebs solution and perfused with 5% CO2 at 36°C. Arterial tone was measured as changes in isometric force. The rings that showed vasoconstriction response induced by 10 µM phenylephrine (PE) were studied. PE was then washed out and the tension was returned to the baseline levels before the administration of the following drugs.

Chemicals and drugs. PKC inhibitor peptide 19–31 (PKC) was purchased from Calbiochem, La Jolla, CA. [Arg8]-vasopressin (acetate salt), pinacidil, glibenclamide, PMA, calphostin-C, [Deamino-Pen1, Tyr(Me)2, Arg8]-vasopressin (YM-AVP), and other chemicals were purchased from Sigma. Chemicals were prepared in high-concentration stock solution in double-distilled H2O or DMSO and were diluted in bath solution to experimental concentrations immediately before usage. In cases where DMSO was used, its concentration was controlled at <0.01% (vol/vol), which did not change the activity of Kir6.1/SUR2B channel. AVP, glibenclamide, pinacidil, and PMA were applied to cells by using a perfusion system. AVP was administered after the maximum current activation by pinacidil was reached. PKCI was included in the pipette solution (10 µM). To avoid ATP degradation, all ATP-containing solutions were made immediately before experiments and were used for no longer than 4 h. Since the variation of Cl− concentrations in solutions was rather small, the resulting liquid junction potential was <1 mV, according to the Henderson equation, and was thereby not corrected.

Data analysis. The concentration-response relationship was fitted with the regular Hill equation: \[ y = 1 / \left( 1 + \left( [AVP] / IC_{50} \right)^h \right) \], where [AVP] is the AVP concentration, h is the Hill coefficient, and IC50 is the [AVP] at midpoint of response inhibition. Data were presented as means ± SE. Differences in means were tested with the ANOVA or Student’s t-test and were accepted as significant if P ≤ 0.05.

RESULTS

Expression of Kir6.1/SUR2B channels in HEK-293 cells. Expression of AVP receptors in HEK-293 cells has been successfully used to identify signal pathways of AVP (15, 34). The Kir6.1/SUR2B channel was transiently expressed in HEK-293 cells. Whole cell patch clamp was performed on GFP-positive cells. The bath solutions contained 145 mM K+ so that the reversal potential of K+ currents is near 0 mV. The recording pipette was filled with the same solution with the addition of 1 mM ATP, 0.5 mM ADP, and 1 mM free Mg2+.
The transfected cells exhibited small baseline currents upon the formation of whole cell configuration (Fig. 1, A and B). An exposure to 10 μM pinacidil increased the currents markedly. The pinacidil-activated currents were strongly inhibited by 10 μM glibenclamide (Fig. 1). These, as well as single-channel properties (see Biophysical mechanisms), were consistent with the characteristics of Kir6.1/SUR2B currents reported previously (33, 39, 43).

Inhibition of Kir6.1/SUR2B channels by AVP. When the V1a receptor was cotransfected with Kir6.1/SUR2B in HEK-293 cells, the currents activated by 10 μM pinacidil were strongly inhibited with an exposure to 100 nM AVP plus 10 μM pinacidil (Fig. 1A). For quantitative analysis, we normalized the affected currents between maximum channel inhibition by 10 μM glibenclamide and maximum activation by 10 μM pinacidil. Evident channel inhibition was seen with 300 pM AVP (16.6 ± 8.1%, n = 8), and stronger inhibition occurred with higher concentrations, with 1 nM (26.4 ± 10.9%, n = 6) and 3 nM (52.2 ± 10.6%, n = 8). The concentration-response relationship can be described by using the Hill equation with IC50 2.0 nM, and (h) 1.0 (Fig. 2B). The maximal inhibition was reached with 10 nM AVP (62.9 ± 10.7%, n = 5). Higher concentration showed slightly further inhibitory effect, with 30 nM AVP (64.0 ± 9.6%, n = 11), 100 nM AVP (66.0 ± 5.4%, n = 8), and 300 nM AVP (66.8 ± 8.6%, n = 6). The Kir6.1/SUR2B currents were also studied in cells transfected without V1a receptor in which no evident inhibition of the Kir6.1/SUR2B currents was seen with 100 nM AVP (Fig. 1B).

Biophysical mechanisms. In whole cell recordings, the currents activated by pinacidil showed almost a linear conductance with no obvious rectification, which was consistent with previous reports (43). The currents inhibited by AVP were isolated by subtracting the remaining currents from the maximum currents activated by pinacidil. When the current-voltage relationship of the currents was plotted with the pinacidil-activated currents, they superimposed almost completely (Fig. 3G), indicating that effect of AVP is not voltage dependent.

In cell-attached patches, currents with single-channel conductance of 39.1 ± 3.3 pS (n = 8) were observed. Exposure of the cells to 10 μM pinacidil increased NPo, from 0.021 ± 0.030 to 0.140 ± 0.072 (Fig. 4). AVP subsequently reduced NPo to 0.037 ± 0.026 (P < 0.01, n = 5). In contrast to NPo, single-channel conductance did not show any significant change in the presence of AVP (38.0 ± 4.7 pS, n = 8) (Fig. 4). Therefore, these results suggest that the inhibition of whole cell Kir6.1/SUR2B currents by AVP is produced by the suppression of the NPo, without affecting the single-channel conductance.

PKC dependence. Previous studies have shown that Kir6.1/SUR2B channel activity is affected by both PKA and PKC (29, 35). The V1a receptor is known to be coupled to G protein Goq activation, which leads to activation of phospholipase C and PKC (13). Therefore, it is possible that the Kir6.1/SUR2B channel inhibition by AVP is mediated by activation of the PKC pathway. To test this hypothesis, we studied the Kir6.1/SUR2B channel by interference with the PKC signaling system. PMA is a potent PKC agonist that anchors PKC to the cellular membrane and persistently exposes its catalytic site (26). Administration of 100 nM PMA reduced the pinacidil-activated currents by 84.5 ± 5.9% (n = 6) (Fig. 5A). In the presence of PMA, 100 nM AVP had no significant inhibitory effect on the currents (8.4 ± 5.1%, n = 6). In contrast, inactive phorbol ester, 4α-phorbol 12,13-didecanoate (4α-PDD) had little inhibitory effect (13.3 ± 8.5%, n = 4), and the whole currents were further inhibited by 100 nM AVP (63.9 ± 9.9%, n = 4) after the cells were pretreated with 4α-PDD (Fig. 5, D and E). With pretreatment with 100 nM calphostin-C, a selective PKC blocker, for 20 min, the inhibitory effect of AVP was significantly diminished (15.4 ± 1.7%, n = 5, P < 0.01).
Fig. 2. Concentration-dependent inhibitions of Kir6.1/SUR2B currents by AVP. A: clear inhibition of the pinacidil-activated currents occurred with 0.3 nM AVP, and a higher concentration (100 nM) of AVP further decreased the whole cell currents. B: effect of AVP was measured and normalized between the maximum channel inhibition by 10 µM glibenclamide and maximum channel activation by 10 µM pinacidil, and was plotted against AVP concentrations. The concentration-response relationship was described using the Hill equation with IC50 = 2.0 nM and Hill coefficient (h) = 1.0.

Fig. 3. Voltage dependence. A–D: whole cell currents were recorded under the same condition as shown in Fig. 1. A series of command pulses were given from −120 mV to 120 mV with 20-mV increments. The voltage protocol did not produce evident currents in the baseline (A). The Kir6.1/SUR2B currents were strongly activated with 10 µM pinacidil (B). Pinacidil-activated currents were inhibited by 100 nM AVP (C) and 10 µM glibenclamide (D). When plotted against membrane potential (Vm), the pinacidil-activated currents showed almost a linear conductance (E). A similar current-voltage relationship was seen after the AVP exposure (F). When the currents inhibited by AVP were isolated with a subtraction of F from E, scaled to the maximum at 120 mV and plotted together with the pinacidil-activated currents, the currents were almost completely superimposed (G).
**Fig. 4.** Effect of AVP on single-channel currents. A–D: single-channel currents were recorded in a cell-attached patch. The lower trace in each panel is an expansion from the record of upper trace between arrows. An active channel was seen at baseline (A). When the cell was exposed to 10 μM pinacidil, the single-channel current was activated with the appearance of a second active channel (B). The currents were strongly inhibited with 100 nM AVP (C). Glibenclamide (10 μM) further inhibited the currents (D). E and F: single-channel conductance was measured in the same cell with a ramp voltage from −100 to 100 mV. Two channels were active in E. Slope conductance of the currents was 38 pS with pinacidil (E) and AVP exposure (F). Note that leak currents were manually removed, and the slope conductance was not measured at baseline because of low appearance of the channel. AVP showed significant suppression of overall channel activity in which the number of openings was not counted (NPo; G) but not single-channel conductance (H). **P < 0.01.

**Fig. 5.** PKC dependence. A: pinacidil-activated currents were markedly inhibited by 100 nM PMA. B: inhibitory effect of AVP was diminished with a 20-min pretreatment of 100 nM calphostin-C. C: in the presence of 10 μM PKC 19–31 in pipette solution, the current response to AVP was also reduced. D: pinacidil-activated currents were not inhibited with a 5-min exposure to 100 nM 4α-phorbol 12,13-didecanoate (4α-PDD), and were inhibited by 100 nM AVP. E: summary of effects of PKC activator on the Kir6.1/SUR2B currents. Data are shown as means ± SE (n = 4 to 8). PDD, 4 α-PDD.
When the pipette solution contained PKCi (10 μM) (Fig. 5, C and E), which acted as a pseudosubstrate by binding to the catalytic site of PKC, 100 nM AVP did not produce any significant channel inhibition (3.2 ± 5.1%, n = 5, P > 0.05) (Fig. 5, C and E). Taken together, all of these results suggest that the Kir6.1/SUR2B channel inhibition by AVP is very likely to be mediated through the PKC system.

**Effects of AVP on endogenous currents of vascular smooth myocytes.** Vascular smooth myocytes (VSMs) were freshly dissociated from the mesenteric arteries (Fig. 6A). Whole cell currents were recorded from these cells under the same condition as for the HEK-293 cells. The VSMs showed small baseline currents, and the current amplitude increased markedly with an exposure to 10 μM pinacidil. The pinacidil-activated currents were strongly inhibited by 100 nM AVP in the presence of pinacidil (by 63.1 ± 5.3%, n = 6) (Fig. 6, B and E).

In cell-attached patches, a K⁺ current with single-channel conductance of ~35 pS was observed in the acutely dissociated VSMs (Fig. 6C). The current had rather low baseline activity with NPo of 0.004 ± 0.002 (n = 4), consistent with a previous report (25). The channel activity increased with an exposure to

![Image](image-url)

**Fig. 6.** Effects of AVP on inward K⁺ currents in vascular smooth myocytes (VSM). A: cells were acutely dissociated from the rat mesenteric artery and photographed before patch-clamp experiments. Calibration = 30 μm. B: whole cell currents were recorded from a dissociated VSM under the same condition as described in Fig. 1. Similar to the Kir6.1/SUR2B channel-expressed HEK-293 cells, the whole cell K⁺ currents were strongly activated with 10 μM pinacidil. Application of 100 nM AVP suppressed the currents in the presence of pinacidil. The currents were further inhibited by 10 μM glibenclamide. Note that individual records are shown (bottom) with time expansion. C: single-channel current was recorded in a cell-attached patch obtained from a dissociated VSM in the same condition as in Fig. 4. The current showed single-channel conductance of 35 pS with ramp voltages from −100 to 100 mV. D: with a membrane potential of −80 mV, the channel activity (C) was low at baseline. The single-channel currents were activated with 10 μM pinacidil. The pinacidil-activated currents were strongly inhibited by 100 nM AVP in the presence of pinacidil. The lower trace in each panel is an expansion from the record of upper trace between arrow heads. E: summary of percentage inhibition of channel activity by 100 nM AVP in whole cell (WC; n = 6) and cell-attached patch (n = 4) configurations. No statistical difference was found, although the inhibition of single-channel currents was lower. Data were obtained from acutely-dissociated VSMs and shown as means ± SE.
10 μM pinacidil and was inhibited with 100 nM AVP in the extracellular solution (Fig. 6, D and E). The pinacidil-activated current had single-channel conductance of 34.8 ± 1.1 pS (n = 4), which did not change significantly with AVP (35.3 ± 0.8 pS, n = 4, P > 0.05). Application of glibenclamide led to a further inhibition of this current. Therefore, these pharmacological properties of this 35-pS current were consistent with our observations in the whole cell recordings from the VSMs, suggesting that the VSM-endogenous KATP channel is inhibited by AVP.

Constriction of mesenteric artery by activation of V1a receptors. AVP produced concentration-dependent constrictions of the isolated mesenteric artery rings with EC50 6.5 nM (Fig. 7, A and B). At the maximum effect, pinacidil relaxed the vasoconstriction almost completely in the continuing presence of AVP, strongly suggesting that the KATP channel is involved (Fig. 6A). A repetitive exposure of AVP in 45 min after the first treatment did not significantly change the reactivity of vascular ring (0.40 ± 0.05 and 0.37 ± 0.05 g, respectively, n = 5 rings from two animals, P > 0.05, Fig. 7C). This effect did not seem to be mediated through endothelium, as AVP continued producing contractions in endothelium-free rings (0.52 ± 0.09 g, n = 3) (see online version of this article for supplemental figure). The effect of AVP was blocked by 30 nM YM-AVP, a selective V1a receptor blocker (Fig. 7, D and E) (0.260 ± 0.002 and 0.028 ± 0.000 g, respectively, n = 4, P < 0.05). The AVP-induced vasoconstriction was attenuated by a preexposure to 1 μM calphostin-C (P < 0.05, n = 4; Fig. 7, E and F). Similar to the effect of AVP, application of PMA (1 μM) produced vasoconstriction (0.41 ± 0.16 g, n = 5) that was relaxed by 10 μM pinacidil (0.05 ± 0.03 g, n = 5 rings from 4 animals).

DISCUSSION

Our results from the present study indicate that the VSM isoform of KATP channels is one of the major targets of AVP. We have found that AVP strongly inhibits the Kir6.1/SUR2B channel expressed in the HEK cell line. The channel inhibition is mediated by selective suppression of NPo without effect on the single-channel conductance. Activation of the V1a receptor and PKC system is crucial for the channel inhibition. Similar effects are also observed in acutely dissociated VSMs. AVP constricts mesenteric arteries where Kir6.1/SUR2B channel is expressed (30, 39), and such a vasoconstriction also depends on the KATP channel, V1a receptor, and PKC pathway.

As an effective vasoconstrictor, AVP is believed to modulate contractions of vascular smooth muscles through multiple ion channels. At physiological concentrations, AVP stimulates Ca2+ spiking in cultured aortic smooth myocytes, which can be abolished by mibefradil at submicromolar concentrations that
do not inhibit L-type Ca\(^{2+}\) currents, suggesting that the T-type, or low-threshold voltage-activated Ca\(^{2+}\) channels, are activated by AVP (4). Another study showed that the L-type Ca\(^{2+}\) channel is also involved in the AVP-induced vasoconstriction (18). In addition to the Ca\(^{2+}\) channels, AVP is known to produce Ca\(^{2+}\) influx through voltage-dependent and receptor-operated cation channels (37), which was recently demonstrated to be the heteromultimeric TRPC6-TRPC7 channel (22). Activation of these voltage-independent Ca\(^{2+}\) channels and cation channels can raise intracellular Ca\(^{2+}\) and initiate contractile filament sliding, when depolarization also occurs simultaneously. The vasoconstrictive effect of AVP depends on extracellular Ca\(^{2+}\) entry in male rat aorta, whereas female aorta depends more upon intracellular Ca\(^{2+}\) released from the sarcoplasmic reticulum. In this regard, gonadal steroid hormones may contribute to aortic vasoconstriction induced by AVP (7). AVP also enhanced sympathetic vasoconstriction at a lower temperature (30\(^{\circ}\)C) by activating V1α receptor (10). However, intracellular signal pathways and target molecules of AVP are still not fully understood. Accumulating experimental evidence indicates that K\(_{ATP}\) channels are inhibited by AVP leading to depolarization. In guinea pig ventricular myocytes, AVP inhibits K\(_{ATP}\) channels through V1α receptors with an IC\(_{50}\) of 15 nM (36). In the RINm5F insulin-secreting cell line, AVP inhibits K\(_{ATP}\) channels that are closed by tolbutamide and opened by diazoxide (21), suggesting that the Kir6.2/SUR1 channel is involved. In cultured coronary arterial smooth muscle cells, AVP inhibits a K\(^+\) current in both outside-out and cell-attached patches, and this effect can be reversed by the K\(_{ATP}\) channel opener nicorandil (38). Our results indicate that the vascular isoform of K\(_{ATP}\) channels Kir6.1/SUR2B is indeed targeted by AVP. The effect of AVP is not limited to the heterologous expression system. We have found that the VSM-endogenous K\(_{ATP}\) current is inhibited by AVP to almost the same degree as the Kir6.1/SUR2B channel expressed in HEK293 cells.

Dumont and Lamontagne (6) reported that the AVP-induced vasoconstriction of aortic rings cannot be blocked by glibenclamide and suggested that K\(_{ATP}\) channels do not play a role in the vasoconstrictive effect of AVP. Since the effect of the K\(_{ATP}\) channel blocker depends on the channel-open state, it may not have significant effect when the channels are closed. When the K\(_{ATP}\) channels are opened by lemakalim, the same study indeed showed a significant attenuation of the AVP-induced vasoconstriction, which is consistent with our observations in the present study. The evidence of glibenclamide alone may not be sufficient to overthrow the contribution of these K\(^+\) channels to the AVP effect, as glibenclamide also affects other channels, such as ROMK and CFTR (16, 19), and glibenclamide may act on thromboxane receptors attenuating indirectly the AVP-induced vasoconstriction of placental chorionic plate arteries (40). The observations that the K\(_{ATP}\) channel openers relax arterial rings in previous reports (6, 38) and that AVP inhibits the Kir6.1/SUR2B and the tissue-endogenous K\(_{ATP}\) channels shown in our current studies strongly suggest that the Kir6.1/SUR2B channel plays, at least in part, a role in the AVP-induced vasoconstriction.

Consistent with the idea that the V1α receptor is the AVP receptor in vascular smooth muscles (11), our studies have shown that YM-AVP, a selective V1 receptor antagonist, blocks the vasoconstriction effect of AVP in mesenteric artery rings. In HEK cells, we have found that Kir6.1/SUR2B currents are inhibited by AVP only when the V1α receptor is coexpressed, indicating that the Kir6.1/SUR2B channel is a downstream effector of the V1α receptor. The V1α receptor is linked to G\(_{q}\), which activates the PKC-dependent intracellular signaling system (2). Several vasoconstrictors, such as angiotensin II, serotonin, and histamine have been shown to inhibit vascular K\(_{ATP}\) channels through the PKC signal pathway (3, 20). Our results suggest that the inhibition of Kir6.1/SUR2B channels by AVP is also mediated via PKC: 1) PMA resembles the inhibitory effect of AVP, while 4\(\alpha\)-PDD has no obvious effect on whole currents; 2) Kir6.1/SUR2B channel inhibition by AVP is abolished in the presence of selective PKC antagonists calphostin-C or PKCi; 3) AVP has no further inhibitory effect when the Kir6.1/SUR2B currents were inhibited by PMA; and 4) PKC dependence is not limited in the HEK cells, as calphostin-C also blocks the vasoconstriction produce by AVP in mesenteric arteries. Furthermore, we have shown that the inhibition of Kir6.1/SUR2B currents by AVP is mediated via suppression of NP\(_{q}\) instead of unitary conductance, consistent with previous studies showing that PKC regulates K\(_{ATP}\) channels through gating mechanism although the PKC phosphorylation sites were still unidentified (28, 35). Therefore, it is possible that the channel-gating mechanisms are targeted by PKC phosphorylation. In addition to direct phosphorylation of the channel protein, the internalization or endocytosis of the V1α receptor and/or Kir6.1/SUR2B channel may be another mechanism for the decrease in functional channel activity, as shown previously for the Kir6.2 channel (14). In our in vitro experimental condition, the low baseline activity of VSM-endogenous K\(_{ATP}\) channel is consistent with a previous report (25). Since the K\(_{ATP}\) channels are strongly activated by several vasodilating hormones through the PKA system (41), it is possible the channel may maintain fair basal activity in vivo, as suggested previously (27). Since how the Kir6.1/SUR2B channel is inhibited by AVP is varied, we believe the PKC pathway is still unclear, the demonstration of the target molecule of AVP and the involvement of PKC should be helpful for further investigations.

AVP is a potent vasoconstrictor that has been known to be useful when systemic circulation loses its reactivity to traditional antishock drugs, such as ephedrine and dopamine during septic shock, a severe infection disease with the overall mortality rate of 49.7% (9). The rationale for the application of AVP is its relative lower plasma concentration (1 pg/ml, 10\(^{-12}\) M) and hypersensitivity to its vasoconstrictor effects during such a pathological condition (24). Therapeutic application of AVP can be even more effective if its target molecules and critical intracellular signal pathways are known. In this regard, our current studies appear to constitute a significant step toward the understanding of vascular regulation by AVP and K\(_{ATP}\) channels.

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