Role of thromboxane \( \text{A}_2 \)-activated nonselective cation channels in hypoxic pulmonary vasoconstriction of rat

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MAINTENANCE OF THE VENTILATION-PERFUSION ratio is critical for optimization of pulmonary gas exchange. Hypoxic pulmonary vasoconstriction (HPV) is a physiological response of pulmonary arterial smooth muscle cells (PASMCs) under hypoxia, pharmacological inhibition of \( \text{K}^+ \) channels does not induce significant contraction in rat pulmonary arteries. Because a partial contraction by thromboxane \( \text{A}_2 \) (TXA\(_2\)) is required for induction of HPV, we hypothesize that TXA\(_2\) receptor (TP) stimulation might activate depolarizing nonselective cation channels (NSCs). Consistently, we found that 5–10 nM U46619, a stable agonist for TP, was dispensible for contraction of rat pulmonary arteries by 4-aminopyridine, a blocker of voltage-gated \( \text{K}^+ \) channel (\( K_c \)). Whole cell voltage clamp with rat PASMC revealed that U46619 induced a NSC current (\( I_{\text{NSC,TXA}_2} \)) with weakly outward rectifying current-voltage relation. \( I_{\text{NSC,TXA}_2} \) was blocked by ruthenium red (RR), an antagonist of the transient receptor potential vanilloid-related channel (TRPV) subfamily, 2-Aminoethoxydiphenyl borate, an agonist for TRPV1–3, consistently activated NSC channels in PASMCs. In contrast, agonists for TRPV1 (capsaicin), TRPV3 (camphor), or TRPV4 (\( \alpha\)-PDD) rarely induced an increase in the membrane conductance of PASMCs. RT-PCR analysis showed the expression of transcripts for TRPV2 and TRPV4 in rat PASMCs. Finally, it was confirmed that pretreatment with RR largely inhibited HPV in the presence of U46619. The pretreatment with agonists for TRPV1 (capsaicin) and TRPV4 (\( \alpha\)-PDD) was ineffective as pretone agents for HPV. Taken together, it is suggested that the concerted effects of \( I_{\text{NSC,TXA}_2} \) activation and \( K_c \) inhibition under hypoxia induce membrane depolarization sufficient for HPV. TRPV2 is carefully suggested as the TXA\(_2\)-activated NSC in rat PASMCs.

hypoxia; pulmonary artery; transient receptor potential

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se, a more direct depolarizing effect of TXA$_2$ (e.g., NSC activation) is supposed, which has not yet been proven. With this background, we revisit the role of TXA$_2$ for HPV and investigate the effects of U46619, a stable analog of TXA$_2$, on membrane conductance of PASMCs.

**MATERIALS AND METHODS**

**Preparation of PAs and isometric tension measurement.** This investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, Revised 1996) and also was reviewed and approved by the IACUC of the Seoul National University College of Medicine. Male Sprague-Dawley rats (250–300 g) were fully anesthetized with pentobarbital sodium (100 mg/kg ip) and killed by cervical dislocation, and their lungs were rapidly moved into a normal Tyrode (NT) solution.

The second- and third-order branches of PAs (diameter: 200–300 μm) were carefully dissected and cut into segments without removing endothelium (length: 2 mm). A segment of artery was mounted on two 25-μm wires in a Mulvany-type myograph (Myo-Interface model 410A; DMT, Aarhus, Denmark), and stabilized in physiological salt solution containing 74% N$_2$, 21% O$_2$, and 5% CO$_2$ at 37°C. Before the experiments, the viability of the arteries was evaluated from the strong contraction in response to 80 mM KCl-PSS (80K contraction). The 80 mM KCl-PSS was prepared by equimolar substitution with NaCl in PSS. To induce hypoxic conditions, the O$_2$ fraction of bubbling gas was lowered to 3% O$_2$ by substitution with N$_2$. Partial pressure of oxygen [PO$_2$ (%)] nearby the PAs was measured using a micro-oxygen electrode (MI-730; Microelectrodes, Bedford, NH) and confirmed that the PO$_2$ (%) was actually lowered to 3%.

**Preparation of single myocytes.** The second- and third-order branches of the intrapulmonary arteries were initially incubated in digestion medium I [Ca$^{2+}$-free NT solution containing papain (1 mg/ml), BSA (1.5 mg/ml), and dithiothreitol (1.5 mg/ml at 37°C for 15 min)]. The arteries were then moved into the digestion medium II [Ca$^{2+}$-free NT solution containing collagenase (2.5 mg/ml), BSA (1.5 mg/ml), and dithiothreitol (1.5 mg/ml) at 37°C for 12 min. Finally, this enzyme-containing solution was washed out by being rinsed with a Ca$^{2+}$-rich free NT solution. The pulmonary arteries were then gently agitated using a wide-bored fire-polished glass pipette and stored in a modified Kraft-Brühe storage solution at 4°C until they were used in the experiments.

**RT-PCR.** Total RNA was prepared from freshly isolated rat PASMCs by TRIzol extraction (Invitrogen, Carlsbad, CA). Specific primers for TRPC1, TRPC6, TRPV1, TRPV2, TRPV3, and GAPDH are shown in Table 1. cDNA were amplified with 2× Taq premix (SolGent, Deageon, Korea) using a DNA thermal cycler (Bio-Rad Laboratories, Hercules, CA). Amplification was performed with 30–35 cycles, as follows: 60°C (1 min), 72°C (2 min), and 95°C (45 s).

**Patch-clamp experiments.** Cells were transferred to a small chamber (0.2 ml) on the stage of an inverted microscope (IX-70; Olympus) and perfused continuously with NT solution at a rate of 10 ml/min. A glass microelectrode with a resistance of 2–2.5 MΩ was used. The conventional whole cell or nystatin perforated patch-clamp technique was used to hold the membrane potential at −60 mV with a patch-clamp amplifier (EPC9; HEKA Electronic, Lambrecht/Pfalz, Germany). Data were filtered at 5 kHz and were analyzed using Origin (ver. 8.0; Microcal Software, Northampton, MA). Either step-like depolarizations or ramp-like pulses were applied to obtain current-voltage relations (I/V) curves of PASMCs. For isolation of the TASK-like background-type K$^+$ current, the cells were clamped at 0 mV for ≥2 min to inactivate voltage-gated K$^+$ channels; reverse ramp pulses (from 60 mV to −100 mV) were then applied to obtain I/V curves.

The bath solution was made hypoxic by bubbling with 100% N$_2$ gas in a separate glass reservoir for ≥20 min before the perfusion. The reservoir was connected to the experimental chamber using oxygen-impermeable Tygon tubing (Saint-Gobain PPL, Seoul, Korea). PO$_2$ (%) was measured in the experimental chamber using the oxymeter (MI-730; Microelectrodes). Upon perfusion with N$_2$-bubbled NT solution, the PO$_2$ (%) of the bath solution was lowered from 21 to ~3%.

**Solutions and chemicals.** Composition of NT solution was as follows (in mM): 143 NaCl, 5.4 KCl, 0.33 NaH$_2$PO$_4$, 1.8 CaCl$_2$, 0.5 MgCl$_2$, 10 HEPES, and 10 glucose (pH 7.4 with NaOH). The pipette solution for recording whole cell K$^+$ current contained the following (in mM): 140 KCl, 5 EGTA, 10 HEPES, and 1 MgCl$_2$ (pH 7.2 with KOH). The pipette solution for nystatin-perforated patch configuration contained the following (in mM): 140 KCl, 10 HEPES, 1 EGTA, 1 MgCl$_2$, and 50 μg/ml nystatin (pH 7.2 with KOH). To change the pH of the bath solution, MES/HEPES NT was prepared by replacement of HEPES with equimolar amounts of MES and HEPES (5 mM, respectively). The pipette solution for recording current I$_{Ca,L}$ consisted of (in mM): 30 CsCl, 110 CsAsp, 10 EGTA, 10 HEPES, and 1 MgCl$_2$ (pH adjusted to 7.25 with CsOH). Also, the CaCl$_2$ in NT solution was substituted with 10 mM BaCl$_2$. K$^+$-rich pipette solution for conventional whole cell clamp was consisted of 30 KCl, 110 KAsp, 10 EGTA, 10 HEPES, and 1 MgCl$_2$ (pH adjusted to 7.25 with KOH).

For recording of NSC current, bath solution consisted of the following (in mM): 140 CsCl, 4 NaCl, 1.8 CaCl$_2$, 0.5 MgCl$_2$, 10 HEPES, and 10 glucose (pH 7.4 with CsOH). The pipette solution for recording NSC current contained the following (in mM): 140 CsCl, 10 HEPES, 1 MgCl$_2$, 4 MgATP, and 5 EGTA (pH 7.2 with CsOH). The composition of Kraft-Brühe storage solution was as follows (in mM):

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Table 1. Nucleotide sequences of the primers used for RT-PCR

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<th>GeneBank No.</th>
<th>Primer</th>
<th>Sequence (5’ to 3’</th>
<th>Expected Size, bp</th>
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<tr>
<td>Rat GAPDH</td>
<td>NM_017008.3</td>
<td>Forward</td>
<td>GCCGGCTTGTTGCGAGGAG</td>
</tr>
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70 KOH, 50 l-glutamic acid, 55 KCl, 20 KH2PO4, 3 MgCl2, 10 glucose, 10 HEPES, and 0.5 EGTA (pH 7.3 with KOH).

The PSS for the myograph experiment consisted of the following composition (in mM): 118 NaCl, 4 KCl, 0.44 NaH2PO4, 24 NaHCO3, 1.8 CaCl2, 1 MgSO4, and 5.6 glucose. Isotonic high K+ solutions (80 mM) were prepared by replacement of NaCl with an equimolar amount of KCl in PSS.

Y-27632 and anandamide were purchased from Tocris (Ellisville, MO). Collagenase was purchased from Wako chemicals (Osaka, Japan). Other drugs including tetraethylammonium (TEA) and 4-AP were obtained from Sigma (St. Louis, MO).

Statistical analysis. Data are presented as the original recordings and bar graphs of the means ± SE (for n tested cells or tissues). Paired or unpaired Student’s t-test was used for statistical analysis, which was accepted at P < 0.05.

RESULTS

First, the indispensability of TXA2 as a pretone agent for HPV was confirmed in rat PAs (Fig. 1A). A strong HPV (101 ± 1.3% of 80K-induced contraction; n = 7) was consistently observed when PAs were pretreated with nanomolar ranges of U46619 (5–10 nM) that alone induced only a slight contraction (5–10% of 80K contraction; Fig. 1D, black bar). Without U46619, no HPV was induced by hypoxia (Fig. 1A, left). HPV was completely reversed by nifedipine, an L-type Ca2+ channel (VOCCl) inhibitor (Fig. 1, B and D; n = 7). In contrast, pretreatment with PKC inhibitor (Gö6976; 100 nM) and RhoA-dependent kinase (ROK) inhibitor (Y-27632; 300 nM) did not result in blockade of HPV (Fig. 1C; n = 3).

Next, we confirmed hypoxic inhibition of K+ channels in PASMCs. In nystatin-perforated whole cell clamp condition, voltage-gated K+ currents (IKv) were elicited by step-like depolarization pulses. Amplitudes of outward currents were decreased by hypoxia (3% PO2), which was more prominent at the later part of the step pulse (Fig. 2, A and B). The reason for stronger inhibition of steady-state IKv is not clear; however, similar time-dependent inhibitions have

Fig. 1. Requirement of thromboxane A2 (TXA2) for hypoxic pulmonary vasoconstriction (HPV) in rat pulmonary arteries (PAs). A: hypoxia alone (3% PO2) did not induce contraction of PA (left). In the presence of 10 nM U46619, hypoxia induced a strong contraction equivalent with the response to high K+ (80K, right). B: HPV was fully reversed by 1 μM nifedipine. C: pretreatment with PKC inhibitor (Gö6976; 100 nM) and RhoA-dependent kinase inhibitor (Y-27632; 300 nM) did not affect HPV in the presence of U46619 (10 nM). D: summaries of HPV normalized to 80K contraction are shown as a bar graph (means ± SE; *P < 0.05, by paired t-test).
been reported in porcine pulmonary artery and rat neuroepithelial bodies (13, 36).

We also examined functional expression of TASK-like current ($I_{\text{TASK}}$) and its O$_2$ sensitivity. To induce inactivation of $I_{\text{Kv}}$, cells were held at depolarized voltage (0 mV) between reverse-ramp pulses (from 60 to $-100$ mV). To further exclude maxi-K and ATP-sensitive K$^+$ channels, 2 mM TEA and 10 nM glibenclamide were included in the bath solution. The slope of current-voltage relation ($I/V$ curve) was decreased by acidic extracellular pH (pHe) with reversal potential close to the equilibrium potential for K$^+$, consistent with the property of $I_{\text{TASK}}$ (Fig. 2, C and E). In addition, the amplitudes of $I_{\text{TASK}}$ were decreased by hypoxia (3% PO$_2$). Anandamide, an inhibitor of the TASK subfamily, largely suppressed the amplitudes of the noninactivating current (Fig. 2 F, open bar).

We then investigated the effects of K$^+$ channel blockers on PA tone and HPV. Neither TEA (2 mM) nor 4-AP (5 mM) increased the tone of PA (Fig. 3 A). Of particular interest, 4-AP induced a strong sustained contraction in the presence of 10 nM U46619, and addition of hypoxic condition induced only a weak further contraction (Fig. 3 B). However, 2 mM TEA induced only a weak contraction even in the presence of U46619, and additional hypoxia (3% PO$_2$) induced typical HPV in a reversible manner (Fig. 3 C). A summary of the above result is shown as bar graphs (Fig. 3 D).

To investigate the role of $I_{\text{TASK}}$ in PASMCs, we performed testing to determine whether HPV or 4-AP-induced contraction could be induced under acidic pHe. However, without U46619, no significant contraction was induced by hypoxia or by 4-AP at pHe 7.0 (Fig. 3 E; n = 6). Different from the effect of 4-AP, application of anandamide did not induce contraction even in the presence of U46619, while HPV was normally observed (data not shown). In addition, partial depolarization by raising extracellular K$^+$ concentration to 25 mM was inefficient as a permissive condition for the HPV of PA (Fig. 3 H). The depolarizing effect of U46619-induced inward current and the positive modulation of $I_{\text{Ca,L}}$ might have been synergistically augmented by the hypoxic inhibition of K$^+$ channels in rat PASMCs. The inadequacy of 25K-induced pretone for HPV responses might owe to the chemical voltage clamp effect; the 25K condition would not allow further depolarization in spite of the hypoxic inhibition of $I_{\text{Kv}}$ and $I_{\text{TASK}}$ in PASMCs.

The results of PA contraction study indicate a critical role of TXA$_2$-induced current and HPV. Therefore we investigate the effects of U46619 on the membrane currents of PASMCs under voltage clamp condition. Interestingly, the application of 1 μM U46619 increased the amplitudes of $I_{\text{Ca,L}}$. 

Fig. 2. Effect of hypoxia on K$_v$ and TWIK-related acidic pH-sensitive K$^+$ channel (TASK)-like current in rat pulmonary arterial smooth muscle cells (PASMCs). A and B: representative current traces recorded in the nystatin-perforated whole cell clamp condition (left). Step-like pulses were applied from $-80$ to 20 mV (20-mV increment, 500-ms duration), and holding potential was at $-60$ mV. Outward currents were decreased by hypoxia (3% PO$_2$). Amplitudes of currents were measured at the later part of voltage pulses and are shown as a bar graph (right, means ± SE). *P < 0.05, by paired t-test. C and D: representative traces of TASK-like current ($I_{\text{TASK}}$). Reverse-ramp pulses from 60 to $-100$ mV were applied with a holding potential of 0 mV. $I_{\text{TASK}}$ was increased by alkalization (pHe 8.4) and decreased by acidification (pHe 6.4). In addition, $I_{\text{TASK}}$ was decreased by hypoxia (3% PO$_2$). E and F: amplitudes of $I_{\text{TASK}}$ measured at 0 mV are summarized. F: inhibition of $I_{\text{TASK}}$ by anandamide (10 μM) is summarized (open bar; *P < 0.05, by paired t-test).
The property of inward conductance induced by U46619 was further investigated by patch-clamp experiment using CsCl pipette solution. Application of 1 μM U46619 resulted in increased membrane conductance with reversal potential at −0 mV (Fig. 5A). Of particular interest, additional hypoxia (Po2, 3%) appeared to further increase the conductance, albeit without statistical significance (Fig. 5A, see also Fig. 4B). While not
Camphor, a TRPV3 agonist, had no effect on cells, Fig. 6. Capsaicin (4 out of 15 cells; Fig. 6) and by 4-PDD (4 out of 15 cells) were used in the pipette and bath (n = 2-APB, 100 μM), to PASMCs. While hypoxic inhibition of K+ current was consistently observed in rat PASMCs, the K+ channel inhibitor alone did not induce vasoconstriction unless isolated PAs had been pretreated with the TXA2 analog U46619. The most intriguing finding was that U46619 activates NSC channels (I_{NSC,TXA2}), the biophysical and pharmacological characteristics of which are indicative of TRPV2.

Finally, we examined the effects of blocker and activators of TRPV on HPV. Pretreatment with 30 μM RR largely abolished HPV in the presence of U46619 (Fig. 7A). Considering the limited selectivity of RR effects on ion channels, we tested RR on 80K-contraction. Although previous study (5) of cloned voltage-gated Ca2+ channels demonstrated the nonspecific inhibition of L-type Ca2+ current (I_{Ca,L}) by RR (5), the 80K-induced contraction of PA was not affected by RR (n = 5; Fig. 7B). While both TRPV1 and TRPV4 activities of rat PASMCs were confirmed in the whole cell clamp studies (Fig. 6, B and D), neither 4α-PDD nor capsaicin was an effective pretone condition for HPV (Fig. 7, C and D).

**DISCUSSION**

In the present study, we investigated the role of TXA2 in HPV with regard to its supposedly depolarizing effects on rat PASMCs. While hypoxic inhibition of K+ current was consistent observed in PASMCs, the K+ channel inhibitor alone did not induce vasoconstriction unless isolated PAs had been pretreated with the TXA2 analog U46619. The most intriguing finding was that U46619 activates NSC channels (I_{NSC,TXA2}), the biophysical and pharmacological characteristics of which are indicative of TRPV2.

The “O2-sensitive K+ channel hypothesis” has been a popular theory of HPV mechanisms. However, as shown in our present study, a partial contraction by TP stimulation using U46619 is indispensible for HPV. Considering the relatively depolarized threshold voltage for K+, a likely scenario would be that the TP agonist induces a partial depolarization to above the threshold voltage for K+. While statistically insignificant, I_{NSC,TXA2} showed a tendency of augmentation by combined hypoxia (Fig. 5A). As a whole, we schematically propose the electrophysiological model of

**Fig. 4. Increase of L-type Ca2+ channels (I_{Ca,L}) and depolarizing inward current induced by U46619 in rat PASMCs.** A: with the use of Cs+ -rich pipette solution and Ba2+ -containing NT solution, whole cell patch clamp was performed. Step-like depolarization from -70 to 10 mV revealed inward current, indicating Ba2+ current through I_{Ca,L}. U46619 (1 μM) increased the amplitude of I_{Ca,L} by -20% as summarized at right. B: with the use of K+-rich pipette solution and NT bath solution, ramp pulses (from -80 to 80 mV) were applied. At left, I/V curves at physiological ranges of membrane voltage (from -60 to -25 mV) are displayed. An application of U46619 increased inward current with right-shift of reversal potential, which appeared to be further increased by additional hypoxia. Summaries of normalized inward current (pA/pF) at 0 mV under each condition are shown as a bar graph (right; *P < 0.05, by paired t-test).
HPV as a “three-step hypothesis”; initial partial depolarization by \( I_{\text{NSC,TXA2}} \), subsequent or concomitant further depolarization by \( K^+ \) channel inhibition by hypoxia, and activation of voltage-gated \( Ca^{2+} \) channels. It has to be noted that the requirement of the initial depolarization could be species and experimental condition dependent. As mentioned in the Introduction and also shown in Fig. 3, an application of 4-AP alone do not induce contraction in PAs from rat and rabbit (3, 5, 30). However, a literature search indicates that PAs from guinea-pig and mouse show contractile response to 4-AP without pretone agents (12, 17, 44).

Here we focus on the electrophysiological influence of TXA2 receptor stimulation; however, previous studies (26, 43) have suggested more direct mechanisms for contraction by TXA2, such as RhoA/ROK and PKC-mediated \( Ca^{2+} \) sensitization. However, the critical role of U46619 for HPV-PA appears to be unaffected by combined treatment with inhibitors of ROK and PKC (Fig. 1C). This result suggested that the influence of the relatively low concentrations of U46619 used in our present study is mostly an electrophysiological one. As for the depolarizing mechanism by TXA2, we cautiously propose activation of NSC as a major mechanism. Interestingly, the amplitude of \( I_{\text{Ca,L}} \) was also increased by U46619, which might partly contribute to the contractile response of PAs. While not confirmed in our present study, an inhibition of \( K^+ \) by TXA2 (7) might also augment depolarization of PASMCs.

Indispensability of TXA2 as pretone condition? Although the critical role of TXA2 pretreatment for HPV is evident in our present study using isolated PAs, the increase of pulmonary arterial pressure by hypoxic ventilation of blood-perfused lungs (V/P lung model) is well observed without TXA2 pre-treatment (32). One could suggest that, in the V/P lung model close to in vivo situations, endogenous TXA2 might provide the pretone conditions for the HPV. In addition to TXA2, other kinds of endogenous agents might also provide the ‘pretone’ or permissive conditions for HPV in vivo. However, another vasoactive agent for PA, serotonin was not an effective pretone conditions for HPV in the rat PAs although serotonin itself induced contraction (data not shown, \( n = 5 \)). Also, the chemical depolarization of PAs by raising the extracellular \( K^+ \) concentration to 25 mM was rarely effective as a pretone condition for HPV (Fig. 3H). Therefore, the application of TXA2 seems to be an efficient and reliable condition for studying HPV and its mechanisms.
Molecular identification of the TXA2-activated NSC. Among the variety of molecular candidates for NSC, the role of TRPC6 in PAs has been consistently suggested in association with the pathophysiological mechanism of pulmonary hypertension and vascular remodeling (21, 22, 39). In addition, Weissmann et al. (42) suggested that TRPC6 is activated by hypoxia, especially under pretreatment with endothelin-1 in mouse PAs. However, the properties of TRPC6 current in mouse PASMCs are different from those of TRPC6 current in rat PASMCs. TRPC6 current shows relatively linear I/V curves, while TRPC6 current is known to show outwardly rectifying voltage dependence. Pharmacological responses and RT-PCR analysis of rat PASMCs indicate expression of TRPV2 and TRPV4 along with TRPC channels (Fig. 6E). We also confirmed that RR, a widely used TRPV antagonist, largely abolished HPV of PA.

In terms of the known characteristics of I/V curves, the TRPV2 current shows a weakly outward rectifying I/V curve similar to that of $I_{NSC,TXA2}$. Agonists for TRPV3, camphor, had no effect on the membrane conductance of PASMCs (Fig. 6C). Although the agonists for TRPV1 or TRPV4 increased the membrane currents (Fig. 6B and D), their pretreatments were totally ineffective as the pretone condition for HPV (Fig. 7, C and D). Overall, these results suggest that TRPV2 might be a possible candidate as NSC$_{TXA2}$, playing a critical role in HPV of rats. However, owing to the lack of reliable experimental tools, it is cautious to conclude TRPV2 as the NSC$_{TXA2}$.

TRPV2 is originally known as a thermosensitive NSC with relatively high threshold temperature ($\sim$50°C) that is not reachable in physiological conditions of lung. There are yet few reports describing the physiological agonists for TRPV2, let alone the signaling pathways for the activation (33). In general, receptor-mediated activation of TRP, especially TRPC family, is thought to be mediated by DAG produced by hydrolysis of phosphatidylinositol 4,5-bisphosphatase via PLC (8). In contrast, TRPV1–4 channels, namely the thermosensitive TRPs, are primarily activated by temperature increase, and their activation of receptors has rarely been reported (2, 10). Further investigation is still requested for elucidation of the molecular identity of NSC$_{TXA2}$ not to speak of the mechanism of NSC$_{TXA2}$ by TP stimulation.

Putative role of TASK-1 in HPV. Consistent with previous reports by Gurney et al. (16), we observed $I_{TASK}$ and its inhibition by hypoxia in rat PASMCs (Fig. 2D). Gardener et al.
suggested that acidic pH induces inhibition of TASK channels and thereby depolarizes the membrane potential in rat pulmonary arteries. However, in our present study, the acidic pH did not increase the PA tone, and even the combined application of 4-AP induced little further contraction in isolated PA (Fig. 3, E and F). Since the acidic condition is generally vasodilatory (37), one should be cautious in interpretation of responses under acidic pH. Similar limitation is also present for the effects of anandamide, an inhibitor of TASK. According to the literature, anandamide not only inhibits TASK-1 channel but also affects other channels; inhibition of L-type Ca$^{2+}$ channel (15) and activation of large-conductance Ca$^{2+}$-activated K$^+$ channels (35). The lack of a selective inhibitor hinders precise evaluation of the role of TASK in HPV-PA. Nanomolar ranges of TXA$_2$ alone induced only a weak contraction of PAs; therefore, the small amplitude of $I_{\text{NSC, TXA}_2}$ appears insufficient to induce strong depolarization of PASMCs. Regarding the circumstantial data, hypoxic inhibition of $I_{\text{TASK}}$ might also contribute to the initial depolarization along with $I_{\text{NSC, TXA}_2}$.

Taken together, the present study newly suggests the role of $I_{\text{NSC, TXA}_2}$ for HPV in rats. In addition to hypoxic inhibition of K$^+$ channels, a prior or concomitant activation of NSC by pretone agent would be critical for sufficient depolarization of rat PASMCs under hypoxia. Based on the pharmacological evidence and $I/V$ curve, we cautiously propose that TRPV2 might be the NSC$_{\text{TXA}_2}$. Precise molecular identification of NSC$_{\text{TXA}_2}$ might provide an intriguing target for modulation of HPV in physiological and pathophysiological conditions.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

REFERENCES


4. C316 TXA2-INDUCED CURRENT AND HYPOXIC PULMONARY VASOCONSTRICTION


