

ORIGINAL ARTICLE

Serotonin contracts the rat mesenteric artery by inhibiting 4-aminopyridine-sensitive Kv channels via the 5-HT_{2A} receptor and Src tyrosine kinase

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Serotonin (5-hydroxytryptamine (5-HT)) is a neurotransmitter that regulates a variety of functions in the nervous, gastrointestinal and cardiovascular systems. Despite such importance, 5-HT signaling pathways are not entirely clear. We demonstrated previously that 4-aminopyridine (4-AP)-sensitive voltage-gated K⁺ (Kv) channels determine the resting membrane potential of arterial smooth muscle cells and that the Kv channels are inhibited by 5-HT, which depolarizes the membranes. Therefore, we hypothesized that 5-HT contracts arteries by inhibiting Kv channels. Here we studied 5-HT signaling and the detailed role of Kv currents in rat mesenteric arteries using patch-clamp and isometric tension measurements. Our data showed that inhibiting 4-AP-sensitive Kv channels contracted arterial rings, whereas inhibiting Ca²⁺-activated K⁺, inward rectifier K⁺ and ATP-sensitive K⁺ channels had little effect on arterial contraction, indicating a central role of Kv channels in the regulation of resting arterial tone. 5-HT-induced arterial contraction decreased significantly in the presence of high KCl or the voltage-gated Ca²⁺ channel (VGCC) inhibitor nifedipine, indicating that membrane depolarization and the consequent activation of VGCCs mediate the 5-HT-induced vasoconstriction. The effects of 5-HT on Kv currents and arterial contraction were markedly prevented by the 5-HT_{2A} receptor antagonists ketanserin and spiperone. Consistently, α -methyl 5-HT, a 5-HT₂ receptor agonist, mimicked the 5-HT action on Kv channels. Pretreatment with a Src tyrosine kinase inhibitor, 4-amino-5-(4-chlorophenyl)-7-(*t*-butyl)pyrazolo[3,4-*d*]pyrimidine, prevented both the 5-HT-mediated vasoconstriction and Kv current inhibition. Our data suggest that 4-AP-sensitive Kv channels are the primary regulator of the resting tone in rat mesenteric arteries. 5-HT constricts the arteries by inhibiting Kv channels via the 5-HT_{2A} receptor and Src tyrosine kinase pathway.

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INTRODUCTION

Serotonin (5-hydroxytryptamine (5-HT)) is a neurotransmitter that regulates a variety of functions in the nervous, gastrointestinal and cardiovascular systems.¹ 5-HT receptors (5-HTRs) mediate 5-HT effects, are highly expressed on pyramidal neurons in the frontal cortex and have been implicated in several mental health disorders, including schizophrenia, anxiety and depression.^{2–5} 5-HT also has an important role in the control of appetite.⁶ Selective 5-HT

reuptake inhibitors are, therefore, widely used to suppress appetite and reduce body weight^{7,8} by increasing 5-HT concentrations in the neuronal synapse.

However, despite such importance, the signaling pathways that mediate 5-HT effects are not entirely clear. The primary targets of 5-HT in the vasculature and neurons are known to be voltage-gated K⁺ (Kv) channels.^{9–13} Kv1.5 channels appear to be functionally linked to 5-HT signaling¹⁴ in the pulmonary artery and to contribute to the pathogenesis of pulmonary

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hypertension.¹⁵ However, it is not clear which channels are more favorable signal transduction targets of 5-HT at physiological concentrations in systemic resistance arteries, which are arteries that are responsible for the modulation of total peripheral resistance. It has been reported that 5-HT inhibits ATP-sensitive K^+ (K_{ATP}) channels in rabbit cerebral and mesenteric arteries.^{16,17} However, the inhibition of K_{ATP} channels by 5-HT was observed while the channels were stimulated with K_{ATP} channel openers such as pinacidil. Thus, the involvement of the K_{ATP} channel in 5-HT signaling under physiological conditions remains unclear. The role of large conductance Ca^{2+} -activated K^+ (BK_{Ca}) or inward rectifier K^+ (K_{ir}) channels in the 5-HT-mediated vasoconstriction is still controversial.^{18,19} We previously reported that 4-aminopyridine (4-AP)-sensitive Kv currents are the primary regulators of resting membrane potential (E_m) in isolated rat mesenteric arterial smooth muscle cells and that these currents are modulated by 5-HT, resulting in E_m depolarization.¹⁰ However, it is not clear whether the 5-HT-induced electrophysiological changes in mesenteric arterial smooth muscle are important in 5-HT-induced vasoconstriction. Therefore, it is necessary to investigate 5-HT signaling and the detailed role of Kv currents in resistance arteries.

Seven major subtypes of 5-HT receptors are currently recognized.^{20,21} Although the primary receptor subtype for 5-HT in the vasculature is 5-HT receptor subtype 2A,²² recent reports show that other subtypes are also able to mediate the vasoconstrictive properties of 5-HT. For example, the effective anti-migraine drugs, triptans, are all known to bind with high affinity to three 5-HTR subtypes: 5-HT_{1B}R, 5-HT_{1D}R and 5-HT_{1F}R. 5-HT_{1B}R mRNA is densely localized within the smooth muscle. The vasoconstrictive properties of triptans are mediated by an action on 5-HT_{1B}R of the smooth muscle and are responsible for potential adverse cardiac events. Therefore, elucidating the mechanism of 5-HT signal transduction and regulation may be of great relevance for the rational design of novel medications.^{23–25}

Recently, c-Src tyrosine kinase was reported to have a critical role in the 5-HT_{2A}R-mediated contraction in the rat aorta.²⁶ c-Src activation was an early and pivotal mechanism in the 5-HT_{2A}R-mediated contraction in the aorta. However, it is not clear whether Src is part of the mechanism linking 5-HTR activation to Kv channel inhibition.

In this study we examined the hypothesis that 5-HT-induced mesenteric arterial contraction is mediated by inhibition of 4-AP-sensitive Kv channels and subsequent E_m depolarization. The responsible 5-HTR subtype(s) and the role of Src tyrosine kinase were also explored using the patch-clamp technique and isometric tension measurements.

MATERIALS AND METHODS

Animals and tissue preparations

All experiments were conducted in accordance with the National Institutes of Health guidelines for the care and use of animals, and the institutional animal care and use committee of the Konkuk University

approved this study. Male Sprague–Dawley rats (10–11 weeks old) were exsanguinated by cutting the carotid arteries under deep ketamine–xylazine anesthesia or after exposure to 100% carbon dioxide. The branches of the superior mesenteric arteries were rapidly isolated and placed in physiological saline solution (PSS) containing 136.9 mM NaCl, 5.4 mM KCl, 1.5 mM $CaCl_2$, 1.0 mM $MgCl_2$, 23.8 mM $NaHCO_3$, 1.2 mM NaH_2PO_4 , 0.01 mM EDTA and 5.5 mM glucose. The arteries were carefully cleaned of fat and connective tissue under a stereomicroscope and then sectioned into rings (3.5 mm in thickness) for the tension measurements. The endothelium was removed mechanically with fine stainless steel wire. Removal of the endothelium was confirmed by the absence of relaxation induced by acetylcholine (10 μ M) after constriction by norepinephrine (1–10 μ M) or 5-HT (1–10 μ M).

Tension measurements

The arterial rings were mounted vertically on two L-shaped stainless steel wires in a 3-ml tissue chamber for tension measurements. One wire was attached to a micromanipulator and the other to an isometric force transducer (FT03; Grass Technologies, West Warwick, RI, USA). Changes in isometric force were digitally acquired at 1 Hz using a PowerLab data acquisition system (AD Instruments, Colorado Springs, CO, USA). Resting tension was set to 0.7–0.8 g using a micromanipulator. After equilibration for 60 min under resting tension in a tissue chamber filled with PSS, the rings were sequentially exposed to 70-mM KCl PSS (10 min) and PSS (15 min) three times for stabilization. A high-KCl (70 mM) PSS was prepared by replacing NaCl with equimolar KCl in PSS. Bath solutions were thermostatically controlled at 37 °C and were continuously saturated with a mixture of 5% CO_2 in 95% O_2 to achieve a pH of 7.4.

Single-myocyte isolation

For the patch-clamp study, single-cell suspensions of mesenteric artery myocytes were prepared as described previously.²⁷ Briefly, the arteries were cut into small pieces and transferred to a digestion solution. The tissue was first digested for 15 min in a Ca^{2+} -free normal Tyrode's (NT) solution containing 1 mg ml⁻¹ papain (Sigma-Aldrich, St Louis, MO, USA), 1 mg ml⁻¹ bovine serum albumin and 1 mg ml⁻¹ dithiothreitol. A second 25-min incubation followed in which papain was replaced by 3 mg ml⁻¹ collagenase (Wako, Osaka, Japan). After enzyme treatment, the cells were isolated by gentle agitation with a fire-polished glass pipette in a Ca^{2+} -free NT solution.

Electrophysiological recording

As our previous report showed that the 5-HT effects on the Kv current and E_m were observed well under the nystatin-perforated patch configuration compared with the conventional whole-cell configuration,¹⁰ membrane currents were recorded under a nystatin-perforated patch configuration as described previously.¹⁰ An Axopatch 200B patch-clamp amplifier and a DigiData 1200 interface (Axon Instruments, Foster City, CA, USA) were used for voltage-clamp and data acquisition, respectively. Membrane current data were digitized using the pClamp 6 software (Axon Instruments) at a sampling rate of 1–10 kHz, low-pass-filtered at 1 kHz and stored on a computer. Voltage pulse generation was also controlled using the pClamp 6 software. The patch pipettes were pulled from borosilicate capillaries (Clark Electromedical Instruments, Pangbourne, UK) using a puller (PP-83; Narishige, Tokyo, Japan). We used patch pipettes with a resistance of 2–4 M Ω when filled with the pipette solution. All experiments were carried out at room temperature (20–25 °C).

Solutions and drugs

An NT solution was used as the bathing solution in the patch-clamp study. The pipette solution contained 135 mM KCl, 5 mM NaCl, 1 mM MgCl₂, 10 mM HEPES, 0.05 mM ethyleneglycol-bis (2-aminoethyl)-N,N,N',N'-tetraacetic acid and 200 μg ml⁻¹ nystatin. The pH was adjusted to 7.2 with KOH. Unless otherwise indicated, all chemicals and drugs were purchased from Sigma-Aldrich. Anpirtoline, BW723C86 and α -methyl 5-HT were obtained from Tocris Bioscience (Bristol, UK). Tetraethylammonium (TEA), BaCl₂, 5-HT, α -methyl 5-HT and anpirtoline were prepared as stock solutions in distilled water, and 4-AP was prepared as a stock solution (0.5 M) in a pH-buffered, glucose-free NT solution and adjusted to pH 7.4 with NaOH. Iberitoxin was also prepared as a stock solution in a pH-buffered, glucose-free NT solution. Nifedipine, glybenclamide, ketanserin, spiperone, BW723C86, PP2 (4-amino-5-(4-chlorophenyl)-7-(*t*-butyl)pyrazolo[3,4-*d*]pyrimidine) and PP3 (4-amino-7-phenylpyrazolo[3,4-*d*]pyrimidine) were prepared as stock solutions in dimethyl sulfoxide. The drugs were diluted in the bathing solution on the day of the experiment. The final concentration of dimethyl sulfoxide was <0.01%, except for BW723C86, during the tension measurement (Figures 5e and f). The final dimethyl sulfoxide concentration, when BW723C86 (from the 10-mM stock solution) was applied during the tension measurement, was up to 1% (Figures 5e and f). Nystatin was dissolved in dimethyl sulfoxide before dilution in the pipette solution.

Statistical analysis

Results are presented as the mean \pm s.e. Paired or independent Student's *t*-tests were used to test for significance as appropriate. A *P*-value < 0.05 was considered statistically significant.

RESULTS

We showed previously that 4-AP-sensitive Kv channels are a major contributor to the resting E_m and that 5-HT inhibits Kv channels, which causes a depolarization of E_m in rat mesenteric arterial smooth muscle cells.¹⁰ The depolarization of E_m in smooth muscle increases the open probability of voltage-gated Ca²⁺ channels (VGCCs), leading to Ca²⁺ influx and vasoconstriction. Therefore, in this study, we first examined whether inhibiting 4-AP-sensitive Kv channels could change the resting tone of the mesenteric artery. Among several types of K⁺ channel inhibitors, only 4-AP evoked a marked concentration-dependent vasoconstriction (Figure 1). TEA, iberitoxin and glybenclamide had no effect on vascular tone (Figure 1). The amount of vasoconstriction evoked by BaCl₂ was minimal compared with the level of vasoconstriction induced by 4-AP. These data indicate that 4-AP-sensitive Kv channels are the K⁺ channel that has the most important role in controlling the resting tone of the mesenteric artery.

We then examined whether E_m depolarization, which is caused by inhibiting Kv channels, is critical for 5-HT-induced vasoconstriction (Figure 2). Under control conditions, 5-HT treatment evoked a concentration-dependent constriction of the mesenteric artery (Figure 2a). In contrast, 5-HT-induced vasoconstriction was markedly (>70%) suppressed when the E_m was depolarized by high KCl (70 mM; Figure 2b) or when the VGCCs were inhibited with nifedipine (1 μM; Figure 2c). In the presence of a combination of high KCl and nifedipine, inhibition of 5-HT-induced vasoconstriction was similar to

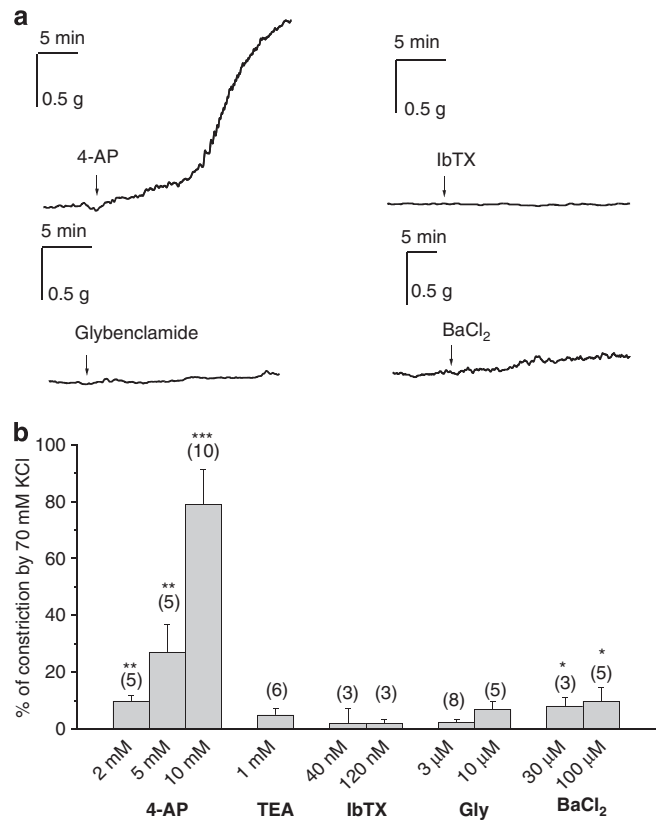


Figure 1 Effects of K⁺ channel inhibitors on the resting tone of mesenteric arterial rings. (a) Typical traces of mesenteric artery constriction responding to the K⁺ channel inhibitor 4-aminopyridine (4-AP, 10 mM), the Ca²⁺-activated K⁺ (BK_{Ca}) channel inhibitor iberitoxin (IbTX, 120 nM), the ATP-sensitive K⁺ (K_{ATP}) channel inhibitor glybenclamide (Gly, 10 μM) and the inwardly rectifying K⁺ (K_{ir}) channel inhibitor BaCl₂ (100 μM). (b) A summary of the vasoconstrictive effects of the K⁺ channel inhibitors. TEA, tetraethylammonium. Numbers in parentheses indicate the number of arterial rings tested. **P* < 0.05, ***P* < 0.01 and ****P* < 0.001 versus resting values.

that with KCl or nifedipine alone (Figures 2d and e). Thus, the effects of KCl and nifedipine were not additive. Taken together, these results suggest that E_m depolarization, which is caused by inhibiting Kv channels, and the consequent activation of VGCCs are critical intermediate steps for vasoconstriction induced by 5-HT.

Next, we determined which of the 5-HTR subtypes were mediating the 5-HT effects in the mesenteric artery. We recorded Kv currents using the nystatin-perforated patch-clamp technique with depolarizing voltage steps as described previously (Figure 3).¹⁰ Under control conditions, 5-HT treatment reduced outward Kv currents (Figures 3a and b). Consistent with the data shown in Figure 1, 5-HT-induced inhibition of the K⁺ current was not affected by the combined treatment of TEA (1 mM), a BK_{Ca} blocker, and glybenclamide (1 μM), a K_{ATP} channel inhibitor (Figures 3c and d). These data support the notion that the currents modulated by 5-HT are 4-AP-sensitive Kv currents. We then examined the effect of

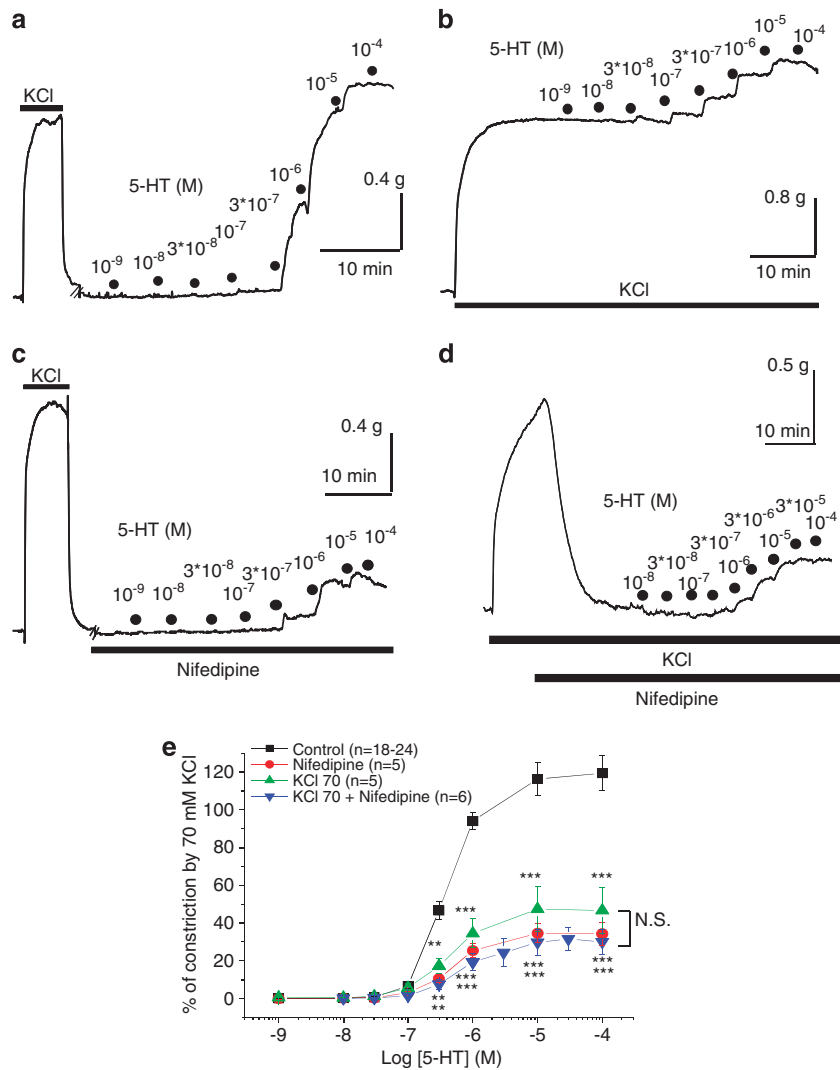


Figure 2 The role of membrane potential (E_m) depolarization and voltage-gated Ca^{2+} channels in 5-hydroxytryptamine (5-HT)-induced mesenteric artery constriction. **(a)** A typical trace of mesenteric artery constriction in response to cumulative concentrations of 5-HT. **(b)** The effect of high KCl (70 mM) pretreatment on 5-HT-induced mesenteric artery constriction. **(c)** The effects of nifedipine (1 μM) on 5-HT-induced constriction. **(d)** The effects of the combined treatment of high KCl (70 mM) and nifedipine (1 μM) on 5-HT-induced constriction. **(e)** Concentration–response curves for 5-HT-induced vasoconstriction under the conditions described in **(a–d)**; both high KCl (70 mM) and nifedipine (1 μM) pretreatment markedly suppressed 5-HT-induced mesenteric artery constriction. High KCl-induced vasoconstriction is shown **(a, c)** before breaks for comparison with 5-HT-induced constriction. The duration of high-KCl treatment was 10 min (note that the timescale bars are for traces after the break). ** $P < 0.01$ and *** $P < 0.001$ versus the control. NS, not significant between all data points between the two groups.

α -methyl 5-HT, a 5-HT₂ receptor agonist, on Kv currents. α -Methyl 5-HT mimicked the 5-HT response, indicating the involvement of 5-HT₂ receptors in the 5-HT-induced vasoconstriction of the mesenteric artery (Figures 3e and f).

To confirm the role of the 5-HT₂ receptors, we examined the effect of ketanserin (100 nM), a competitive 5-HT_{2A} receptor-specific antagonist. Ketanserin abolished the 5-HT-induced inhibition of the K^+ current (Figures 4a and b). Spiperone (10 nM), a more selective 5-HT_{2A} receptor antagonist than ketanserin, also prevented the inhibitory effect of 5-HT on the Kv current (Figure 4c and d). Accordingly, ketanserin (10–100 nM) attenuated 5-HT-induced vasoconstriction

(Figure 4e). Ketanserin shifted the concentration–response curve of 5-HT-induced vasoconstriction to the right in a concentration-dependent manner (Figure 4f). These results suggest that the 5-HT_{2A} receptor mediates both the 5-HT-induced K_v current inhibition and the vasoconstriction in the rat mesenteric artery. We ruled out the possible involvement of 5-HT_{2B} and 5-HT_{1B} receptors with their specific agonists BW723C86 (1 μM) and anpirtoline (1 μM), respectively (Figure 5). Neither 5-HT_{2B} nor 5-HT_{1B} receptor agonists had any effect on the outward K^+ currents (Figures 5a–d) or on constriction of the mesenteric arteries (Figures 5e and f).

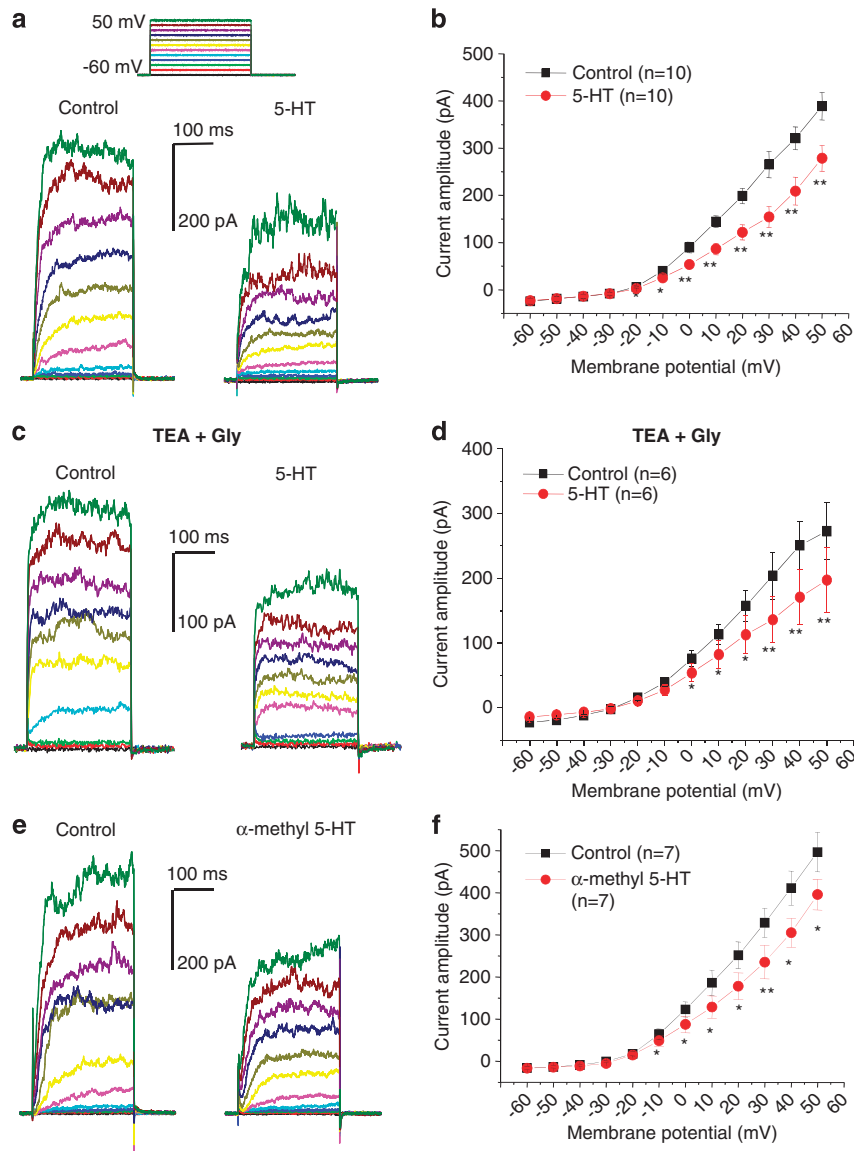


Figure 3 The effects of 5-hydroxytryptamine (5-HT) and α -methyl 5-HT on voltage-gated K⁺ (Kv) currents. (a, e) Representative recordings of K⁺ currents in the absence and presence of 5-HT (1 μ M) or α -methyl 5-HT (1 μ M). The shape of the voltage pulse protocol used to elicit the Kv currents is shown as a figure inset. (b, f) Current-voltage (*I-V*) relationships in the absence and presence of 5-HT (1 μ M) or α -methyl 5-HT (1 μ M). 5-HT and α -methyl 5-HT reduced Kv currents to similar degrees. (c, d) The effect of tetraethylammonium (TEA; 1 mM) plus glybenclamide (1 μ M) pretreatment on the inhibiting effect of 5-HT on the K⁺ currents. **P* < 0.05 versus the control. ***P* < 0.01 versus the control.

Recently, 5HT_{2A}Rs have been reported to be coupled with the activation of Src tyrosine kinase in the aorta.²⁶ To determine whether Src tyrosine kinase contributes to the 5-HT_{2A}R-mediated Kv channel inhibition and contraction in the rat mesenteric artery, we examined the effect of the Src kinase inhibitor PP2 on the 5-HT-induced mesenteric arterial contraction and Kv current inhibition. Pretreatment with PP2 (5 μ M) markedly suppressed the mesenteric arterial contraction induced by 5-HT treatment (Figure 6a). Specifically, at a 5-HT concentration below 3 μ M, PP2 almost completely abolished the 5-HT-induced arterial contraction. Moreover, PP2 also completely blocked the 5-HT (1 μ M)-induced inhibition of the

Kv current (Figure 6b and c). However, PP3 (5 μ M), a negative analogue of PP2, did not affect the 5-HT-induced vasoconstriction (Figure 6a) or the Kv current inhibition (Figure 6d and e).

DISCUSSION

The results of the present study suggest that Kv currents are the primary regulator of the resting *E*_m and vascular constriction in the rat mesenteric artery. 5-HT treatment constricted the artery by inhibiting the 4-AP-sensitive Kv currents through the 5-HT_{2A}R. Src tyrosine kinase mediated the 5-HT_{2A}R-induced inhibition of the Kv current and vasoconstriction.

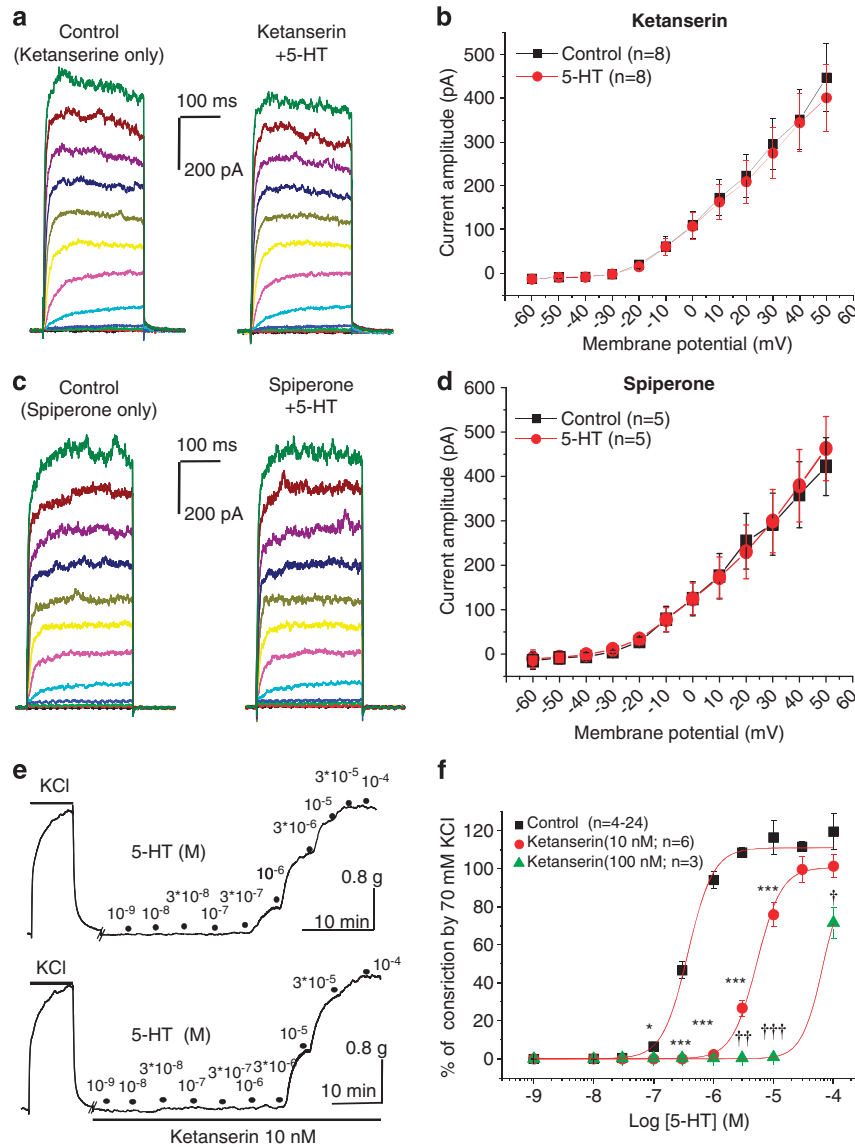


Figure 4 The effects of ketanserin and spiperone, selective 5-hydroxytryptamine (5-HT)_{2A} receptor inhibitors, on the 5-HT-induced inhibition of voltage-gated K⁺ (Kv) currents and vasoconstriction. **(a)** Representative recordings of the Kv currents of ketanserin (100 nM)-pretreated smooth muscle cells in the absence and presence of 5-HT (1 μM). Ketanserin alone had no effect on the Kv currents (data not shown). **(b)** Summary of the I–V relationships of the ketanserin-pretreated cells in the absence and presence of 5-HT (1 μM). **(c)** Representative recordings of the Kv currents of the spiperone (10 nM)-pretreated cells in the absence and presence of 5-HT (1 μM). Spiperone alone had no effect on the Kv currents (data not shown). **(d)** Summary of the I–V relationships of the spiperone-pretreated cells in the absence and presence of 5-HT (1 μM). **(e)** Typical traces of mesenteric artery constriction in response to cumulative concentrations of 5-HT in the absence (upper panel) and presence (lower panel) of ketanserin (10 nM). **(f)** Concentration–response curves for 5-HT-induced vasoconstriction in the absence and presence of ketanserin (10 or 100 nM). Ketanserin blocked both 5-HT-induced Kv current inhibition and vasoconstriction. High-KCl (70 mM)-induced vasoconstrictions are shown in **e** before breaks for comparison with 5-HT-induced constrictions. The duration of high-KCl treatment was 10 min in all instances (note that the timescale bars are for traces after the break). * $P < 0.05$ and *** $P < 0.001$ versus the control. † $P < 0.05$, †† $P < 0.01$ and ††† $P < 0.001$ versus the ketanserin (10 nM) group.

Roles of 4-AP-sensitive Kv channels and E_m in the resting and 5-HT-induced vascular tone of the rat mesenteric artery

In our previous studies we demonstrated that agents that block the Kv channels, such as 4-AP and ketamine, markedly depolarize the E_m of isolated rat mesenteric arterial smooth muscle cells,^{10,28} suggesting that the 4-AP-sensitive Kv currents may regulate the resting tone of the artery under physiological

conditions. In accordance with this expectation, the present study verified that 4-AP treatment contracted rat mesenteric arterial rings in a concentration-dependent manner (Figure 1). In contrast, other K⁺ channel blockers, such as TEA, ibertoxin, glybenclamide and BaCl₂, had little effect on mesenteric artery contraction. These results indicate that only the Kv channels, not the BK_{Ca}, K_{ATP} or K_{ir} channels, are potential

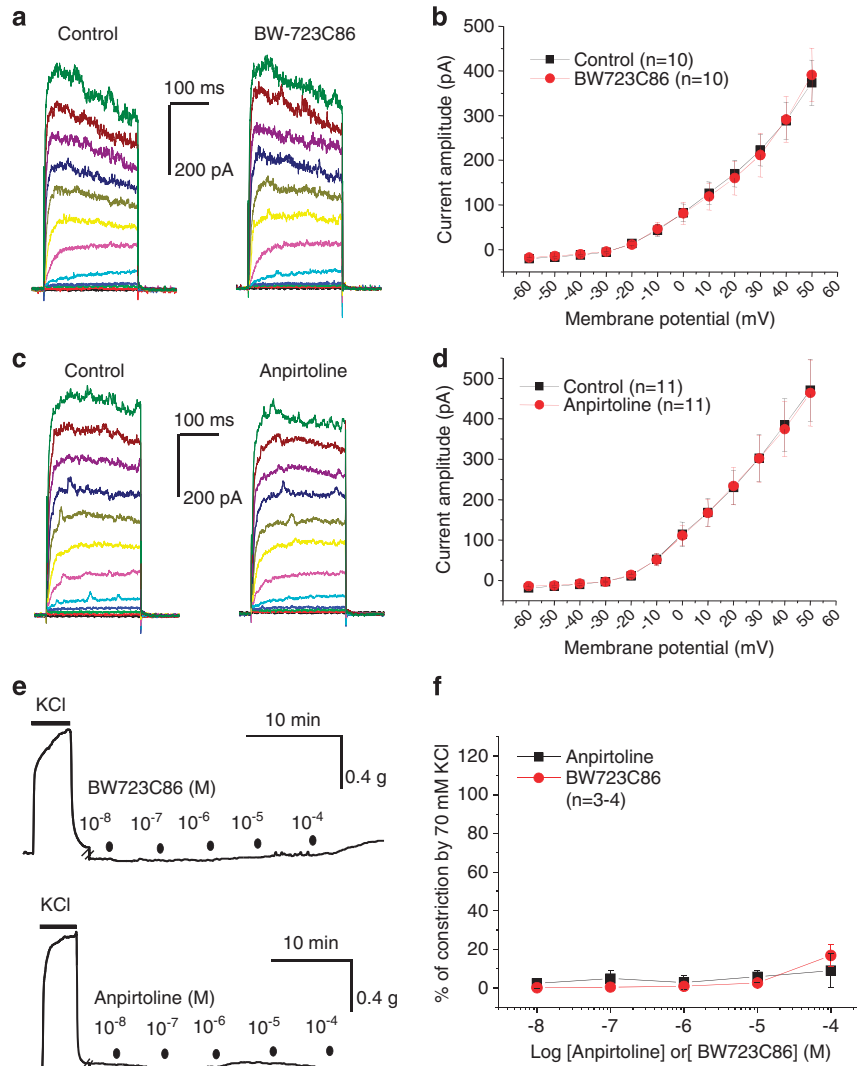


Figure 5 The effects of BW723C86 and anpirtoline on voltage-gated K⁺ (Kv) current and vasoconstriction. **(a, c)** Representative recordings of K⁺ currents in the absence and presence of BW723C86 (1 μM; a 5-HT_{2B} agonist) and anpirtoline (1 μM; a 5-HT_{1B} agonist). **(b, d)** I-V relationships in the absence and presence of BW723C86 (1 μM) and anpirtoline (1 μM). **(e)** The effects of cumulative concentrations of BW723C86 and anpirtoline. **(f)** The summary of **e**. High KCl (70 mM)-induced vasoconstrictions are shown in **e** before breaks for comparison with agonist-induced vasoconstrictions. The duration of high-KCl treatment is 10 min in all instances (note that the timescale bars are for traces after the break).

targets of the vasoconstrictor 5-HT under resting conditions in the rat mesenteric artery. Moreover, the similar inhibition of K⁺ currents in the presence of TEA and glybenclamide (Figure 3) compared with the control condition support the hypothesis that 4-AP-sensitive and TEA/glybenclamide-insensitive Kv currents have a major role in the 5-HT-induced vasoconstriction. However, it is still possible that other K⁺ channels may contribute to the 5-HT-mediated contraction, as the contribution of K⁺ channels is different depending on the artery type and animal species. Moreover, the contributions of BK_{Ca} or K_{ATP} channels can increase when the artery is prestimulated with certain hormones or other neurotransmitters such as calcitonin gene related peptide (CGRP).^{16,17} It is possible that the endothelium may alter the basal contribution of K⁺ channels in smooth muscle cells under physiological

conditions. This contribution warrants future study, because the present study only examined endothelium-denuded arteries. It is also possible that the contributions of ion channels can be altered under pathological conditions. For example, an additional contribution of ion channels other than Kv channels, such as transient receptor potential channels, has been suggested as a mechanism of vascular hyper-responsiveness or increased proliferation, which is found in some pulmonary and general hypertension cases.²⁹⁻³² In addition to Kv channel inhibition, the activation of some inward currents may also mediate 5-HT-induced E_m depolarization and consequent vasoconstriction. As arterial smooth muscle cells usually have very high input resistance or very small input conductance (>GΩ), the activation of even a tiny inward current may induce significant E_m depolarization. The activation of transient

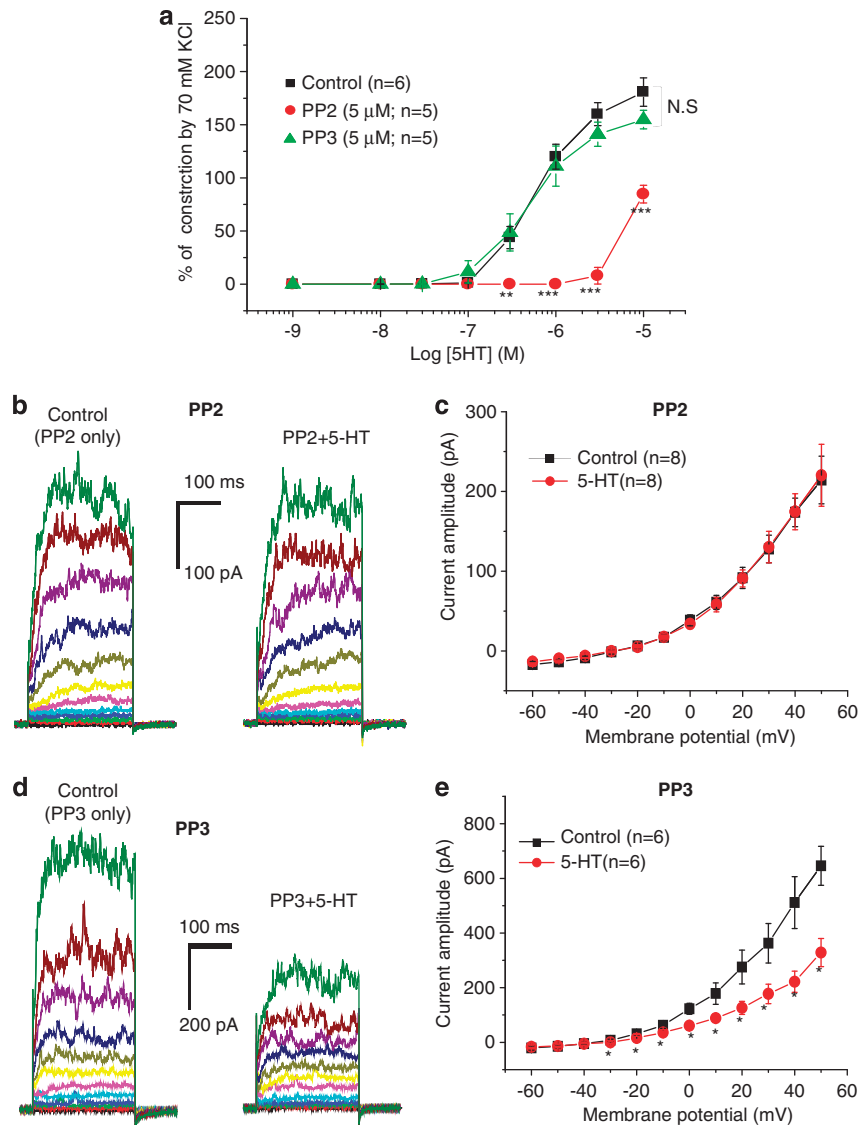


Figure 6 The effects of PP2 (4-amino-5-(4-chlorophenyl)-7-(*t*-butyl) pyrazolo[3,4-*d*]pyrimidine) on the 5-hydroxytryptamine (5-HT)-induced vasoconstriction and Kv current inhibition. **(a)** Concentration–response curves for 5-HT-induced vasoconstriction in the absence and presence of PP2 (5 μM) or PP3 (4-amino-7-phenylpyrazolo[3,4-*d*] pyrimidine; 5 μM). **(b)** Representative recordings of Kv currents of the PP2 (5 μM)-pretreated smooth muscle cells with or without 5-HT (1 μM). **(c)** Summary of the *I*–*V* relationships of the ketanserin-pretreated cells in the absence and presence of 5-HT (1 μM). **(d)** Representative recordings of Kv currents of the PP3 (5 μM)-pretreated cells with or without 5-HT (1 μM). **(e)** Summary for the *I*–*V* relationships of the PP3-pretreated cells in the absence and presence of 5-HT (1 μM).

receptor potential channel-like inward non-selective cation currents by 5-HT was demonstrated in deoxycorticosterone acetate-salt hypertensive rat mesenteric arteries.²⁹ However, the measurement of input resistance by injecting repetitive current, according to the method of our previous study,²⁸ indicated that applying 5-HT did not increase the input conductance of rat mesenteric arterial smooth muscle cells (unpublished observation), supporting the hypothesis that the inhibition of Kv channels, not the activation of some inward cation current, is the primary 5-HT E_m depolarization mechanism in the rat mesenteric artery.

Although some E_m -independent mechanisms, such as protein kinase C activation, Ca^{2+} sensitization of contractile proteins, Ca^{2+} influx through voltage-independent Ca^{2+}

channels or Ca^{2+} release from intracellular stores, clearly contribute to agonist-induced vasoconstriction, increasing evidence supports the hypothesis that sustained E_m depolarization is important in agonist-induced or receptor-mediated vasoconstriction.^{14,28,33,34} Our results (Figure 2) also support the hypothesis that 5-HT constricts the rat mesenteric artery via E_m depolarization: vasoconstriction induced by 5-HT and high KCl was just minimally additive (Figure 2; summarized in Figure 2e). Moreover, nifedipine (1 μM), a specific inhibitor of VGCCs, potently suppressed 5-HT-induced vasoconstriction (>70% inhibition; Figure 2). Simultaneous pretreatment with both high KCl and nifedipine suppressed the 5-HT contraction similar to the contraction suppression by either treatment alone. These results indicate that sustained E_m depolarization

and subsequent Ca^{2+} influx through VGCCs have a major role in the 5-HT-induced vasoconstriction of the rat mesenteric artery.

The 5-HT_{2A}R subtype has a major role in 5-HT-induced Kv channel inhibition and vasoconstriction in the rat mesenteric artery

By using specific inhibitors and agonists for 5-HTRs, we demonstrated that the molecular target of 5-HT in the mesenteric artery is 5-HT_{2A}R. Our data show that 5-HT-induced inhibition of Kv channels was mimicked by α -methyl 5-HT, a 5-HT₂ receptor agonist, and was prevented by selective antagonists of 5-HT_{2A}R, such as ketanserin and spiperone (Figure 4). Consistently, 5-HT-induced vasoconstriction was inhibited by ketanserin (Figure 4). In contrast, selective 5-HTR subtype agonists for 5-HT_{2B} and 5-HT_{1B} had no effect (Figure 5). These findings indicate that the activation of 5-HT_{2A}R mediates the inhibition of the Kv channels and accounts for 5-HT-induced mesenteric arterial vasoconstriction.

The major role of 5-HT_{2A}R in 5-HT-induced mesenteric arterial contraction in the present study was consistent with that of a previous study.³⁵ However, other types of 5-HTRs have also been proposed to mediate the cardiovascular effects of 5-HT. 5-HT_{1B}R mediates the vasoconstrictive properties of triptans, such as sumatriptan, which are used for the treatment of migraine. The vasoconstriction of the coronary artery by tegaserod seems to be mediated via 5-HT_{1B}R.³⁶ Tegaserod is a 5-HT₄R agonist and a promising drug for the treatment of irritable bowel syndrome.³⁷ However, tegaserod was withdrawn from the US market due to its side effect of coronary arterial spasm and heart ischemia.³⁸ Moreover, 5-HT_{1B}R and 5-HT_{2B}R have a key role in deoxycorticosterone-salt hypertensive animal arteries.³⁹ Considering these findings together with those in our present study, it is possible that 5-HT_{2A}R is involved in the 5-HT signaling pathway under control conditions and that other 5-HTRs may be important in pathological conditions. A comprehensive understanding of the molecular features of 5-HT signaling under control conditions promises to identify pathological changes in cardiovascular diseases, such as hypertension, and to advance our knowledge of vascular hyper-responsiveness-associated pathophysiological processes.

Src tyrosine kinase has a critical role in the 5-HT_{2A}R-mediated inhibition of the Kv current and consequent vasoconstriction in the rat mesenteric artery

As we have shown in Figure 5 and have discussed above, 5-HT_{2A}R mediates the 5-HT action in the rat mesenteric artery. Lu *et al.*²⁶ reported that Src tyrosine kinase is a critical component in the 5-HT_{2A}R-mediated contraction in the rat aorta.²⁶ In the present study we provide the evidence that Src tyrosine kinase is responsible for the 5-HT_{2A}R-mediated inhibition of the Kv current in the rat mesenteric artery (Figure 6). Moreover, 5-HT increased the phosphorylation level of Src, as evidenced by western blot analysis (unpublished observation). These observations suggest that 5-HT_{2A}R, Src

tyrosine kinase and the Kv channel pathway have an important role in the 5-HT effect in rat arteries (at least the aorta and small mesenteric artery). Although the molecular identity of the 4-AP-sensitive Kv current in rat arteries is not entirely clear, heteromultimers of Kv1.x channels, such as Kv1.2 and Kv1.5, have been suggested to be largely responsible for the native Kv current.^{40,41} Cogolludo *et al.*¹⁴ reported that in the pulmonary artery, Kv1.5 was a target of 5-HT_{2A}R.¹⁴ The authors demonstrated that tyrosine kinase has a role in the 5-HT_{2A}R-induced inhibition of Kv1.5.¹⁴ These results indicate that Kv1.x channels, including Kv1.5, might be targets for Src tyrosine kinase after 5-HT_{2A}R activation in arteries. The inhibition of Kv1 channels, such as Kv1.2 and Kv1.5, by tyrosine phosphorylation was also demonstrated in human embryonic kidney 293 cells and rat atrial myocytes.^{42,43} In addition to the arteries, 5-HT_{2A}R is highly expressed in the brain, such as in the forebrain region, where this receptor has been implicated in schizophrenia, depression and psychotomimetic effects of hallucinogens.^{2-5,44} Modulations of Kv1 channels by 5-HT_{2A}R are thought to be correlated with these symptoms in the central nervous system (reviewed by D'Adamo *et al.*⁴⁴). For example, 5-HT and toxins that inhibit Kv1.x channels, such as 4-AP and α -dendrotoxin, similarly induce excitatory postsynaptic currents (EPSCs) preferentially in layer V neocortical pyramidal neurons compared with layer II/III or VI neurons.⁴⁴⁻⁴⁶ Taken together, we suggest that a '5-HT_{2A}R-Src tyrosine kinase-Kv1 channel' pathway may be a potential target for treatments of neurophysiological and psychiatric disorders, as well as cardiovascular diseases such as hypertension.

In conclusion, our results suggest that suppressing 4-AP-sensitive Kv channels is a critical intermediate step in the vasoconstrictive actions of 5-HT in the rat mesenteric artery. We also demonstrated that only 5-HT_{2A}R, not 5-HT_{1B}R or 5-HT_{2B}R, mediates the 5-HT effects. Src tyrosine kinase has a critical role in the 5-HT_{2A}R-mediated inhibition of the Kv current and vasoconstriction in the rat mesenteric artery. The results provided here will be useful for a future comparison of 5-HT signaling in arteries between physiological and disease conditions such as hypertension.

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