Anorexic effect of K⁺ channel blockade in mesenteric arterial smooth muscle and intestinal epithelial cells

SHARON S. MCDANIEL,* OLEKSANDR PLATOSHYN,* YING YU, MICHELE SWEENEY, VICTOR A. MIRIEL, VERA A. GOLOVINA, STEFANIE KRICK, BETHANY R. LAPP, JIAN-YING WANG, AND JASON X.-J. YUAN

Department of Medicine, University of California School of Medicine, San Diego, California 92103; Department of Pharmacology and Therapeutics, University of Florida College of Medicine, Gainesville, Florida 32610; and Departments of Physiology and Surgery, University of Maryland School of Medicine, Baltimore, Maryland 21201

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McDaniel, Sharon S., Oleksandr Platosyn, Ying Yu, Michele Sweeney, Victor A. Miriel, Vera A. Golovina, Stefanie Krick, Bethany R. Lapp, Jian-Ying Wang, and Jason X.-J. Yuan. Anorexic effect of K⁺ channel blockade in mesenteric arterial smooth muscle and intestinal epithelial cells. J Appl Physiol 91: 2322–2333, 2001.—Activity of voltage-gated K⁺ (Kv) channels controls membrane potential (Em). Membrane depolarization due to blockade of K⁺ channels in mesenteric artery smooth muscle cells (MASMC) should increase cytoplasmic free Ca²⁺ concentration ([Ca²⁺]cyt) and cause vasoconstriction, which may subsequently reduce the mesenteric blood flow and inhibit the transportation of absorbed nutrients to the liver and adipose tissue. In this study, we characterized and compared the electrophysiological properties and molecular identities of Kv channels and examined the role of Kv channel function in regulating Em in MASMC and intestinal epithelial cells (IEC). MASMC and IEC functionally expressed multiple Kv channel α- and β-subunits (Kv1.1, Kv1.2, Kv1.3, Kv1.4, Kv1.5, Kv2.1, Kv4.3, and Kv9.3, as well as Kvβ1.1, Kvβ2.1, and Kvβ3), but only MASMC expressed voltage-dependent Ca²⁺ channels. The current density and the activation and inactivation kinetics of whole cell Kv currents were similar in MASMC and IEC. Extracellular application of 4-aminopyridine (4-AP), a Kv-channel blocker, reduced whole cell Kv currents and caused Em depolarization in both MASMC and IEC. The 4-AP-induced Em depolarization increased [Ca²⁺]cyt in MASMC and caused mesenteric vasoconstriction. Furthermore, ingestion of 4-AP significantly reduced the weight gain in rats. These results suggest that MASMC and IEC express multiple Kv channel α- and β-subunits. The function of these Kv channels plays an important role in controlling Em. The membrane depolarization-mediated increase in [Ca²⁺]cyt in MASMC and mesenteric vasoconstriction may inhibit transportation of absorbed nutrients via mesenteric circulation and limit weight gain.

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resulting in increased mesenteric vascular resistance to the blood flow. This ultimately may limit the transportation of absorbed nutrients to other tissues for storage.

Furthermore, the Na⁺-dependent uptake of glucose (42), amino acids (36), and long-chain free fatty acids (4, 5, 37) from the lumen of the intestine into intestinal epithelial cells (IEC) is driven by the transmembrane Na⁺ concentration gradient and the Eₘ. Therefore, membrane depolarization (when Eₘ becomes less negative inside the plasma membrane) due to dysfunctional K⁺ channels in IEC may also inhibit the Na⁺-driven absorption of glucose, amino acid, and long-chain fatty acid in the intestine by reducing the transmembrane driving force for Na⁺.

K⁺ channels are ubiquitously expressed in almost all excitable or nonexcitable cells. There are several types of K⁺ channels described in vascular smooth muscle cells and epithelial cells (13, 21, 23, 27, 34, 43). Pharmacological blockade of voltage-gated K⁺ (Kv) channels with 4-aminopyridine (4-AP) induces membrane depolarization, increases [Ca²⁺]ᵢ, and causes vasoconstriction, suggesting that the activity of Kv channels regulates Eₘ under resting conditions in smooth muscle cells (14, 15, 23, 33, 34, 44). It has been demonstrated that the anorexics drugs, fenfluramine and dexfenfluramine, in addition to inhibiting serotonin transporters (2), decrease Kv channel activity in vascular smooth muscle cells (19, 24, 40). These observations suggest that the activity of Kv channels in MASMC may play an important role in the regulation of energy intake by controlling nutrient transportation. In this study, we tested the hypothesis that blockade of Kv channels causes mesenteric vasoconstriction, which should lead to a reduction in nutrient transportation, and therefore ingestion of a Kv-channel blocker inhibits weight gain. To this end, we characterized the electrophysiological properties and molecular identities of Kv channels in MASMC and IEC and investigated the effect of Kv⁺ channel blockade on mesenteric contractility and the anorexic effect of oral ingestion of the K⁺ channel blocker 4-AP in rats.

**MATERIALS AND METHODS**

**Cell preparation and culture.** Primary cultures of MASMC were prepared from Sprague-Dawley rats (44, 45). The third to fourth divisions of mesenteric arterial branches of the abdominal aorta were isolated and incubated for 20 min in Hank's balanced salt solution with 1.5 mg/ml collagenase (Worthington). After the incubation, a thin layer of adventitia was carefully stripped off and endothelium was removed by gently scratching the intimal surface. The remaining smooth muscle was digested with 1.75 mg/ml collagenase and 0.5 mg/ml elastase (Sigma Chemical) for 45 min at 37°C. The cells were plated onto 25-mm coverslips in petri dishes (for electrophysiological and fluorescent experiments) or directly onto 10-cm petri dishes (for molecular biological experiments) and cultured in DMEM containing 10% fetal bovine serum (FBS) in a 37°C, 5% CO₂, humidified incubator. The cellular purity of MASMC in primary cultures was confirmed by the specific monoclonal antibody raised against smooth muscle α-actin (32). Primary cultured cells were first stained with the membrane-permeable nucleic acid stain 4',6'-diamidino-2-phenylindole (DAPI, 5 μM, Molecular Probes) to estimate total cell numbers in the cultures. All the DAPI-stained cells also cross-reacted with the smooth muscle α-actin antibody, indicating that the cultures contained only smooth muscle cells.

The rat IEC-6 cell line was purchased from the American Type Culture Collection at passage 13. Stock cells were maintained in T-150 flasks in DMEM supplemented with 5% FBS, 10 μg/ml insulin, and 50 μg/ml gentamicin sulfate (Sigma Chemical). Flasks were incubated at 37°C in a humidified atmosphere of 5% CO₂ in air. Stock cells were subcultured once a week at 1:20 and plated at 3,500 cells/cm² on coverslips (for electrophysiological and fluorescence experiments) and 10-cm petri dishes (for molecular biological experiments) in DMEM supplemented with 5% dialyzed FBS. Passages 15–17 were used in the experiments. There were no significant changes in biological function and characterization from passages 15–17.

**Electrophysiological measurements.** Whole cell K⁺ currents were recorded with an Axopatch-1D amplifier and a Digidata 1200 interface (Axon Instruments) using patch-clamp techniques (18, 44, 45). Patch pipettes (2–4 MΩ) were made on a Sutter electrode puller using borosilicate glass tubes and were fire polished on a Narishige microforge. Step-pulse protocols and data acquisition were performed by using pCLAMP software. Currents were filtered at 1–2 kHz (–3 dB) and digitized at 2–4 kHz with the use of the amplifier. All experiments were performed at room temperature (22–24°C). Eₘ in single MASMC or IEC was measured in current-clamp mode (I = 0) using the patch-clamp technique.

For recording K⁺ currents, a coverslip containing the cells was positioned in the recording chamber (~0.75 ml) and superfused (2–3 ml/min) with the standard extracellular (bath) physiological salt solution (PSS). The PSS contained (in mM) 141 NaCl, 4.7 KCl, 1.8 CaCl₂, 1.2 MgCl₂, 10 HEPES, and 10 glucose, buffered to pH 7.4 with 5 M NaOH or 2 M Tris. In Ca²⁺-free PSS, CaCl₂ was replaced by equimolar MgCl₂ and 0.1 mM EGTA was added to chelate residual Ca²⁺. The internal (pipette) solution for recording whole cell K⁺ currents contained (in mM) 125 KCl, 4 MgCl₂, 10 HEPES, 10 EGTA, 5 Na₂ATP, buffered to pH 7.2 with 2 M Tris. 4-AP (Sigma Chemical) was directly dissolved into PSS on the day of use; the pH value of the solution was readjusted to 7.4.

**Measurement of [Ca²⁺]ᵢ.** In single MASMC or IEC, [Ca²⁺]ᵢ was measured by using the Ca²⁺-sensitive fluorescent indicator fura 2 and a digital imaging fluorescent microscopy system (16, 17). Cells were loaded with the acetoxymethyl ester form of fura 2, fura 2-AM (3 μM) for 30 min (Molecular Probes), in the dark at room temperature (22–24°C) under an atmosphere of 5% CO₂-95% air. The fura 2-loaded cells on coverslips were then transferred to a recording cell chamber mounted on the microscope stage and superfused with PSS for 20–30 min to remove the extracellular fura 2, which may have diffused out of the cell after initial loading, and to allow cytosolic esterases to cleave fura 2-AM into active fura 2. Subsequent experiments were carried out at 32°C. Fura 2 fluorescence (510-nm light emission excited by 360- and 380-nm illuminations) from the cell, as well as background fluorescence, was imaged using a GEN III charge-coupled device camera (Stanford Photonics) coupled to a Carl Zeiss microscope. Fluorescent images were obtained by using a microchannel plate image intensifier (Amperex XX1381) coupled by fiber optics to the charge-coupled device camera. Image acquisition and analysis were performed with a MetaMorph Imaging System (Universal Imaging). Video
frames containing images of fura 2 fluorescence from cells, as well as the corresponding background images (fluorescence from fields devoid of cells), were digitized at a resolution of 512 horizontal × 480 vertical pixels and at eight-bit gray scale by using a Matrix LC imaging board operating in an IBM-compatible personal computer. To improve the signal-to-noise ratio, four to eight consecutive video frames were usually averaged at a video frame rate of 30 frames/s. Images were acquired at a rate of one averaged image every 3 s when \([\text{Ca}^{2+}]_{\text{cyt}}\) was changing, and every 60 s when \([\text{Ca}^{2+}]_{\text{cyt}}\) was stable. \([\text{Ca}^{2+}]_{\text{cyt}}\) was calculated from fura 2 fluorescence emission excited at 380 and 360 nm by using the ratio method (13). In most experiments, multiple (6–10) cells were imaged in single field, and one arbitrarily chosen peripheral cytosolic area (4–6 × 4–6 pixels) from each cell was spatially averaged (16).

**Measurement of diameter in isolated mesenteric artery.** Isolated mesenteric arteries were dissected by methods similar to those described previously (25). The mesenteric arcade was dissected from the abdominal cavity, cleaned free of blood and connective tissue, and mounted in a temperature-controlled perfusion chamber containing a dissection solution of the following composition (in mM): 145 NaCl, 5 KCl, 2.5 CaCl\(_2\), 1 MgSO\(_4\), 1 K\(_2\)HPO\(_4\), 3 MOPS, 5 glucose, and 1.0% albumin (pH 7.4). The dissected arteries were transferred to a perfusion chamber that was filled with cold Krebs buffer solution. The perfusion chamber housed two glass pipettes filled with Krebs buffer. The artery was cannulated at both ends by the glass pipettes (tip diameter, 60–80 μm) and secured by 10-0 surgical suture. One pipette was attached to a pressure-regulating device (Living Systems) while the other was attached to a closed stopcock. This configuration allowed for the study of pressure-induced responses in the absence of intraluminal flow. The chamber was placed on the stage of an inverted microscope and continuously superfused with Krebs solution (at 37°C), which contained (in mM) 138 NaCl, 4.7 KCl, 1.2 MgSO\(_4\), 1.2 NaH\(_2\)PO\(_4\), 5 HEPES, 1.8 CaCl\(_2\) and 10 glucose (pH 7.4). In Ca\(^{2+}\)-free solution, CaCl\(_2\) was replaced by equimolar MgCl\(_2\) and 0.1 mM EGTA was added to chelate residual Ca\(^{2+}\). In high-K\(^+\) solution, NaCl was replaced by equimolar KCl to maintain osmolality.

**RT-PCR.** Total RNA was prepared from MASMC and IEC by the acid guanidium thiocyanate-phenol-chloroform extraction method and reverse-transcribed using random hexamers (pd(N\(_6\)) primer) (39). The sense and antisense primers were specifically designed from coding regions of the target channels (Table 1). The fidelity and specificity of the sense and antisense oligos were examined by use of the BLAST program. PCR was performed by a GeneAmp PCR system using AmpliTaq DNA polymerase and accompanying buffers. The first-strand cDNA reaction mixture (3 μl) was used in a 50-μl PCR reaction consisting of 0.2 nmol of each primer, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2 mM MgCl\(_2\), 200 μM each 2-deoxynucleotide 5′-triphosphate, and 2 units Taq DNA polymerase. The cDNA samples were amplified in a DNA thermal cycler under the following conditions: the mixture was annealed at 52–61°C (1 min), extended at 72°C (2 min), and denatured at 94°C (1 min) for 20–30 cycles. This was followed by a final extension at 72°C (10 min) to ensure complete product extension. The PCR products were electrophoresed through a 2% agarose gel, and amplified cDNA bands were visualized by ethidium bromide staining.

**Determination of body weight.** Sprague-Dawley rats (125–150 g) were housed in an environmentally controlled room with a 12:12-h light-dark cycle and given ad libitum access to water and Teklad rodent diet (Harlan) containing a minimum of 24% crude protein, 4% crude fat, and a maximum of 4.5% crude fiber. Body weights of rats were measured using a scale in the morning. For the fenfluramine experiment, rats were divided into two groups: the control group \((n = 22)\) in which saline was administered intragastrically daily for 14 days and the fenfluramine-treatment group \((n = 22)\) in which fenfluramine (25 mg/kg, Sigma Chemical) was administered intragastrically once a day for 14 days via a curvedavage needle. For the 4-AP experiment, rats were divided into two groups: the control group \((n = 23)\) was given drinking water with no drug added, and the 4-AP group \((n = 24)\) was given drinking water including 4-AP (2 mM, Sigma Chemical). Drugs were dissolved in drinking water on the day of use; pH values of the solutions were measured and readjusted to the pH of control water. Water bottles for both groups were changed every 2 days for 14 days.

**Statistical analysis.** The composite data are expressed as means ± SE. Statistical analyses were performed by using unpaired and paired Student’s *t*-test or ANOVA and post hoc tests (Student-Newman-Keuls) when appropriate. Differences were considered to be significant when *P* < 0.05.

**RESULTS**

**Electrophysiological properties of Kv channels in MASMC and IEC.** K\(^+\) efflux through Kv channels greatly contributes to the regulation of \(E_m\) under resting conditions in smooth muscle cells (15, 26, 29, 34, 44). Whereas \([\text{Ca}^{2+}]_{\text{cyt}}\) is increased or cellular metabolism (or intracellular ATP content) is decreased, Ca\(^{2+}\)-activated and ATP-sensitive K\(^+\) channels participate in regulating \(E_m\) (27, 34). In the following experiment, Ca\(^{2+}\)-activated and ATP-sensitive K\(^+\) channel currents were minimized by removal of extracellular and intracellular Ca\(^{2+}\) (plus 1–10 mM EGTA) and

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

<table>
<thead>
<tr>
<th>Standard Names (Accession No.)</th>
<th>Predicted Size, bp</th>
<th>Sense/Antisense Location, nt</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Kv channels</strong></td>
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<td></td>
</tr>
<tr>
<td>α-Subunits</td>
<td></td>
<td></td>
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<tr>
<td>Kv1.1 (X12589)</td>
<td>594</td>
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<td>322</td>
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<tr>
<td>Kv1.5 (M27158)</td>
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<td>Kvβ3 (X76723)</td>
<td>395</td>
<td>5′-GGCTAGTATGCTGAGTGC-3′/1683–1685</td>
</tr>
</tbody>
</table>

Kv channels, voltage-gated K⁺ channels; VDCC, voltage-dependent Ca²⁺ channels. Accession numbers in GenBank for the sequences used in designing the primers are shown in parentheses.

inclusion of 5 mM ATP in the intracellular (pipette) solution (27) to focus on the electrophysiological and pharmacological properties of Kv channels in MASMC and IEC.

Whole cell Kv currents \( I_{K(V)} \) in MASMC and IEC were elicited by depolarizing the cells from a holding potential of \(-70 \, \text{mV} \) to a series of test potentials ranging from \(-40 \) to \(+80 \, \text{mV} \) (Fig. 1, A and B). The voltage threshold for \( I_{K(V)} \) activation was between \(-50 \) and \(-40 \, \text{mV} \); the currents at \(-40 \, \text{mV} \) were \( 25.8 \pm 6.7 \, \text{pA} / \text{pF} \) in MASMC and \( 3.8 \pm 0.2 \, \text{pA} / \text{pF} \) in IEC (Fig. 1A). The amplitudes of \( I_{K(V)} \) at \( 40 \, \text{mV} \) were \( 25.8 \pm 6.7 \, \text{pA} / \text{pF} \) and \( 0.6 \, \text{nS} \) in IEC (Fig. 1B). The averaged slope conductances of \( I_{K(V)} \) elicited by a 20-s test pulse of \(+60 \, \text{mV} \) (from a holding potential of \(-70 \, \text{mV} \) in MASMC and IEC were composed of at least three components: a rapidly inactivating component, a slowly inactivating component, and a nonactivating component. The fast and slowly inactivating components of the outward \( I_{K(V)} \) were activated at potentials between \(-60 \) and \(-40 \, \text{mV} \) and completely inactivated at potentials more positive than \(-2 \, \text{mV} \) (Fig. 3B). The half-activation potentials were \(-30 \) and \(-40 \, \text{mV} \), whereas the half-inactivation potentials were \(-12.5 \) and \(-15 \, \text{mV} \), respectively, in MASMC and IEC (Fig. 3B). The window currents appeared to be at a range between \(-50 \) and \(-10 \, \text{mV} \) and peaked at \(-21 \, \text{mV} \) in MASMC and \(-27 \, \text{mV} \) in IEC, suggesting that all the components of \( I_{K(V)} \) participate in the regulation of resting \( E_m \) in these cells. These results indicate that the electrophysiological properties (e.g., current density, voltage threshold for current activation, and kinetics of current inactivation) of \( I_{K(V)} \) are comparable between MASMC and IEC, suggesting
and reversibly reduced whole cell extracellular application of 5 mM 4-AP significantly blocked of Kv channels (3, 23, 27, 44). Indeed, that both cell types functionally express similar Kv channels.

Molecular identification of $I_{K(v)}$ in MASMC and IEC. At the molecular level, Kv channels are homomorphic or heteromeric tetramers composed of the pore-forming α subunits and the cytosolic regulatory β-subunits (12, 20, 21). Using RT-PCR analysis, we identified 1) seven Kv channel α-subunits, Kv1.1, Kv1.2, Kv1.3, Kv1.4, Kv1.5, Kv2.1, and Kv4.3; 2) an electrically silent α-subunit, Kv9.3; and 3) three β-subunits, Kvβ1.1, Kvβ2.1, and Kvβ3, in MASMC and IEC (Fig. 4). The Kv channel α-subunit, Kv1.6, appeared to be expressed only in IEC but not in MASMC (Fig. 4A, middle). These results indicate that both MASMC and IEC express multiple Kv channel α- and β-subunits.

Effects of 4-AP on whole cell $I_{K(v)}$ and $E_m$ in MASMC and IEC. At doses of $\leq 5$ mM, 4-AP is a relatively specific blocker of Kv channels (3, 23, 27, 44). Indeed, extracellular application of 5 mM 4-AP significantly and reversibly reduced whole cell $I_{K(v)}$ in MASMC and IEC (Fig. 5). The 4-AP-sensitive components of whole cell $I_{K(v)}$, revealed by subtracting the currents recorded during application of 4-AP from the currents recorded under control conditions, were activated at potentials more negative than $-40$ mV with slope conductances of $3.1 \pm 0.5$ nS in MASMC ($n = 8$) and $1.7 \pm 0.4$ nS in IEC ($n = 9, P < 0.001$) between $-40$ and 0 mV (Fig. 5, A and B, insets).

Under resting conditions, the membrane input resistance ($R_m$) was very high in MASMC ($9.5 \pm 4.4$ GΩ) and IEC ($8.1 \pm 3.6$ GΩ; $P = 0.84$). Therefore, a small change in $I_{K(v)}$ would cause a large change in $E_m$ in both MASMC and IEC. Resting $E_m$ in MASMC ($-45 \pm 2$ mV, $n = 24$) and IEC ($-39 \pm 2$ mV, $n = 34$) were slightly but not significantly different ($P = 0.36$) under control conditions. Consistent with the inhibitory effect on whole cell $I_{K(v)}$, extracellular application of 5 mM 4-AP reversibly depolarized MASMC by $\sim 16$ mV and IEC by $-18$ mV (Fig. 6A). Similar to the effect of 4-AP, increasing extracellular K+ concentration from 4.7 to 50 mM (which reduces K+ efflux and shifts the K+ equilibrium potential from $-83$ to $-25$ mV) also depolarized $E_m$ in MASMC and IEC (Fig. 6B). These results indicate that activity of Kv channels greatly contributes to the regulation of resting $E_m$ in both MASMC
and IEC and that pharmacological blockade of Kv channels with 4-AP induces membrane depolarization. Effects of 4-AP-induced \(E_m\) depolarization on \([Ca^{2+}]_{cyt}\) in MASMC and on vasomotor tone in isolated mesenteric arteries. Because of the voltage dependence of VDCC and Na\(^+\)/Ca\(^{2+}\) exchanger in the plasma membrane, \(E_m\) serves as a major regulator of \([Ca^{2+}]_{cyt}\) in smooth muscle cells (8, 9, 14, 26, 31, 33). Using RT-PCR, we detected high mRNA levels of VDCC \(\alpha_1\)- and \(\beta_1\)-subunits in MASMC (Fig. 7). In contrast, neither the \(\alpha_1\)- nor the \(\beta_1\)-subunit of VDCC was detected in IEC (Fig. 7), even when the cDNA samples were amplified for more than 35 cycles. These results suggest that VDCC are highly expressed in MASMC but not expressed in IEC. In smooth muscle cells, the voltage window for sustained elevation of \([Ca^{2+}]_{cyt}\) through VDCC ranges from \(-40\) to \(-25\) mV (14, 26, 33). Thus the 4-AP-induced \(E_m\) depolarization would open VDCC and increase \([Ca^{2+}]_{cyt}\). Indeed, blockade of Kv channels with 4-AP induced membrane depolarization (Fig. 6A) and reversibly increased \([Ca^{2+}]_{cyt}\) (Fig. 8, A and B) in MASMC.

In vascular smooth muscle cells, a rise in \([Ca^{2+}]_{cyt}\) is a major trigger for vasoconstriction (35). Indeed, the 4-AP-induced membrane depolarization and increase in \([Ca^{2+}]_{cyt}\) caused mesenteric vasoconstriction. In isolated mesenteric arterial segments, extraluminal application of 2 mM 4-AP reduced the diameter of the pressurized artery by \(-20\%\) (Fig. 8, C and D). Furthermore, 10 mM tetraethylammonium, a K\(^+\) channel blocker, also caused significant vasoconstriction; the diameter of the vessels was reduced from \(125 \pm 3\) to \(99 \pm 6\) \(\mu\)m (\(n = 6, P < 0.01\)). Because vascular resistance is inversely proportional to the fourth power of the arterial lumen radius, the 4-AP-induced mesenteric vasoconstriction would be expected to signifi-
Significantly increased mesenteric vascular resistance to the blood flow.

Effects of 50 mM K\(^+\) on [Ca\(^{2+}\)]\(_{cyt}\) in MASMC and IEC. Raising extracellular K\(^+\) concentration to 50 mM depolarized \(E_m\) in both MASMC and IEC (Fig. 6B). In MASMC that highly express VDCC, the 50 mM K\(^+\)-induced membrane depolarization significantly
increased \([\text{Ca}^{2+}]_{\text{cyt}}\) (Fig. 9A). Removal of extracellular \(\text{Ca}^{2+}\) abolished the high \(\text{K}^{+}\)-mediated increase in \([\text{Ca}^{2+}]_{\text{cyt}}\) (data not shown), suggesting that the \([\text{Ca}^{2+}]_{\text{cyt}}\) is increased mainly by \(\text{Ca}^{2+}\) influx through opened VDCC in MASMC. Accordingly, extracellular application of 50 mM \(\text{K}^{+}\)-containing solution irreversibly blocked the high \(\text{K}^{+}\)-induced vasoconstriction (Fig. 9B).

Because VDCC are not expressed in IEC, the membrane depolarization (Fig. 6B) induced by raising extracellular \(\text{K}^{+}\) concentration to 50 mM negligibly affected \([\text{Ca}^{2+}]_{\text{cyt}}\) (Fig. 9A). Actually, the membrane depolarization due to inhibited Kv channels attenuated \(\text{Ca}^{2+}\) influx through voltage-independent \(\text{Ca}^{2+}\) channels (e.g., store-operated \(\text{Ca}^{2+}\) channels) (28) in IEC by reducing the transmembrane driving force for \(\text{Ca}^{2+}\) (41).

Anorexic effect of blocking Kv channels by 4-AP on weight gain in rats. Because the mesenteric blood flow is required to transport absorbed nutrients from intestine to liver and adipose tissues, an increase in the mesenteric vascular resistance would lead to a decrease in nutrient transportation (10, 11) and may thus inhibit weight gain.

Body weight of the rats in the control group (\(n = 22–26\)), fed with water and normal food, rose by 55–85 g after 2 wk. Fenfluramine (25 mg/kg body wt), an appetite suppressant that has been demonstrated to block Kv channels in vascular smooth muscle cells (24), reduced weight gain in rats by 64% compared with the rats intragastrically administered with saline (Fig. 10A). Similarly, the Kv-channel blocker 4-AP (2 mM, dissolved in the drinking water) inhibited weight gain in rats by 60% compared with the rats administered only drinking water (87.3 ± 7.8 vs. 35.1 ± 7.5 g, \(P < 0.001)\) (Fig. 10B).

DISCUSSION

Body weight or fat accumulation in adipose tissues is controlled by a balance of energy (food) intake and expenditure. A major function of mesenteric circulation is to transport absorbed products of digestion (e.g., glucose, amino acids, and fatty acid) to liver and adipose tissues for synthesizing triacylglycerols, an important form of accumulated fat. The transportation of the absorbed nutrients is directly proportional to the mesenteric blood flow, which mainly depends on the contractile state of mesenteric arteries. Food intake or exposure of intestinal epithelium to glucose increases mesenteric blood flow due to nitric oxide-mediated mesenteric vasodilation (10, 11), suggesting a significant contribution of enhanced mesenteric vasodilation to the transportation of absorbed nutrients to other

Fig. 8. Blockade of Kv channels with 4-AP increases cytoplasmic free \(\text{Ca}^{2+}\) concentration (\([\text{Ca}^{2+}]_{\text{cyt}}\)) and induces mesenteric vasoconstriction. A: representative record of \([\text{Ca}^{2+}]_{\text{cyt}}\) in a MASMC (left) and summarized data (means ± SE, right) showing \([\text{Ca}^{2+}]_{\text{cyt}}\) measured in peripheral areas of MASMC (\(n = 64\)), before (basal), during (4-AP), and after (wash) application of 5 mM 4-AP. ***\(P < 0.001\) vs. basal. B: pseudocolor \(\text{Ca}^{2+}\) images (top panels) showing \([\text{Ca}^{2+}]_{\text{cyt}}\) in the cells before, during, and after extracellular application of 4-AP, and a fura 2 fluorescence (\(F_{\text{380}}\)) image (bottom panel) showing the cells from which \([\text{Ca}^{2+}]_{\text{cyt}}\) was measured. C: schematic diagram showing the setup used to measure vessel diameter. The vessel (a) was cannulated by glass pipettes (b) and was superfused with Krebs solution in a recording chamber (c) on the microscope. The 2 vertical white broken lines in the transmitted light image (d) of a pressurized rat mesenteric artery are the video calipers used to measure lumen diameter. D: representative record (left) of the lumen diameter of an isolated rat mesenteric artery before and during application of 4-AP (2 mM). Summarized data (means ± SE, right) showing the averaged diameters of mesenteric arteries (\(n = 8\)) before (cont) and during (4-AP) 3-min treatment with 4-AP. **\(P < 0.01\) vs. control.
tissues. Therefore, a reduction in mesenteric blood flow would diminish the transportation of absorbed nutrients to liver and adipose tissues and may inhibit weight gain.

In this study, we observed that MASMC and IEC similarly expressed multiple Kv channels. The whole cell $I_{\text{Kv}}$ shared similar electrophysiological (e.g., current density, voltage threshold, kinetics of current activation and inactivation) and pharmacological (e.g., in response to 4-AP) properties in these two cell types. Inhibition of Kv channels by 4-AP or decrease of $K^+$ efflux by raising extracellular $K^+$ concentration to 50 mM induced membrane depolarization in both MASMC and IEC. Because MASMC highly express VDCC, the 4-AP- or 50 mM $K^+$-induced membrane depolarization reversely increased $[Ca^{2+}]_{\text{cyt}}$, caused mesenteric vasoconstriction, and reduced the diameter of mesenteric artery. The resultant decrease in the blood flow through mesenteric arterioles and capillaries would ultimately inhibit the transportation of absorbed nutrients to other tissues. In IEC, the 50 mM $K^+$-mediated $E_m$ depolarization had little effect on $[Ca^{2+}]_{\text{cyt}}$ because VDCC are not expressed in these cells. However, the membrane depolarization would reduce the transmembrane $Na^+$ driving force required for the $Na^+$-dependent uptake of glucose, amino acid, and long-chain free fatty acid in epithelial cells (4, 5, 36, 37, 42) and thus may contribute to inhibit absorption of the nutrients in small intestine. Indeed, 4-AP, similar to the appetite suppressant fenfluramine, significantly reduced weight gain in rats.

**Role of $K^+$ channel activity in the regulation of $E_m$ in MASMC and IEC.** In both excitable and nonexcitable cells, the transmembrane $K^+$ permeability through Kv channels is a key determinant of $E_m$ when the $K^+$ gradient remains constant (27). It has been demonstrated that $K^+$ currents through Kv channels are, in part, responsible for controlling $E_m$ under resting conditions in smooth muscle cells (11, 19, 24, 33). Blockade of Kv channels (e.g., by 4-AP) induces $E_m$ depolarization because of decreased $I_{\text{Kv}}$, whereas opening of Kv channels. 

**Fig. 9. Effects of 50 mM $K^+$ on $[Ca^{2+}]_{\text{cyt}}$ in MASMC (left) and IEC (right) and on isometric tension in isolated mesenteric arterial rings.** A: representative records (a) showing time courses of the 50 mM $K^+$-induced $[Ca^{2+}]_{\text{cyt}}$ changes in MASMC and IEC. Summarized data (b, means ± SE) showing $[Ca^{2+}]_{\text{cyt}}$ levels before (basal), during (50 K), and after (wash) extracellular application of 50 mM $K^+$ in MASMC (gray bars, n = 45) and IEC (solid bars, n = 35). ***$P < 0.001$ vs. basal and wash. B: representative record showing time course of the 50 mM $K^+$-induced contraction in an isolated mesenteric arterial ring (a). $Ca^{2+}$-free solution (0 Ca) containing 50 mM $K^+$ was applied to the vessel when 50 mM $K^+$-mediated contraction reached plateau. Summarized data (b, means ± SE, n = 16) showing vessel tension before (basal), during (50 K), and after (wash) application of 50 mM $K^+$ in the presence (1.8Ca) or presence (0 Ca) of extracellular $Ca^{2+}$ (1.8 mM). ***$P < 0.001$ vs. 0Ca.

**Fig. 10. Inhibition of Kv channels with 4-AP reduces body weight gain in rats.** A: summarized data (means ± SE) showing weight gain in rats intragastrically fed with saline (control, ○, n = 22) or fenfluramine (Fen, 25 mg/kg, ●, n = 22). ***$P < 0.001$ vs. control (Student-Newman-Keuls). B: summarized data (means ± SE) showing weight gain in rats that drank water (control, ○, n = 23) or water containing 4-AP (2 mM, ●, n = 24). ***$P < 0.001$ vs. control (Student-Newman-Keuls).
channels (e.g., by nitric oxide) induces \( E_m \) hyperpolarization because of increased \( I_{K(V)} \) (22, 47). Both MASMC and IEC have very large membrane input resistance, and thus a very modest change in whole cell \( I_{K(V)} \) would cause a large change in \( E_m \).

In MASMC and IEC, we identified 1) seven functional Kv channel \( \alpha \)-subunits (Kv1.1, Kv1.2, Kv1.3, Kv1.4, Kv1.5, Kv2.1, and Kv4.3), 2) an electrically silent Kv channel modulatory \( \alpha \)-subunit (Kv9.3), and 3) three Kv channel \( \beta \)-subunits (Kv\( \beta \)1.2, Kv\( \beta \)2.1, and Kv\( \beta \)3). Additionally, Xu et al. (43) recently demonstrated that MASMC also express Kv2.2, Kv3.2, Kv3.3, Kv3.4, Kv4.1, and Kv4.2. The Kv channel \( \alpha \)-subunit, Kv1.6, was the only subunit tested in our study that was highly expressed in IEC but not in MASMC (also see Ref. 43). These observations suggest that whole cell \( I_{K(V)} \) are controlled by homomeric and heteromeric channels encoded by multiple Kv channel \( \alpha \)- and \( \beta \)-subunits. Which Kv channel subunits dominantly contribute to the regulation of \( E_m \) in MASMC and IEC is still incompletely understood.

**Role of \( E_m \) in the regulation of mesenteric vascular resistance.** A rise in \( \left[ Ca^{2+}\right]_{\text{cyt}} \) in vascular smooth muscle cells is a major trigger for vasoconstriction (35). \( \left[ Ca^{2+}\right]_{\text{cyt}} \) can be increased by \( Ca^{2+} \) influx through sarcolemmal \( Ca^{2+} \) channels and by \( Ca^{2+} \) release from intracellular stores (mainly the sarcoplasmic reticulum) (6–9, 38). By governing \( Ca^{2+} \) influx via VDCC, \( E_m \) plays a critical role in regulating \( Ca^{2+} \) and vascular tone (26, 38). In smooth muscle cells, much of the increase in cytosolic \( Ca^{2+} \) necessary for muscle contraction is due to \( Ca^{2+} \) entry through plasma membrane \( Ca^{2+} \) channels, dominantly VDCC (14, 26, 33, 35, 44). Because MASMC highly express VDCC, the 4-AP- or 50 mM \( K^+ \)-induced \( E_m \) depolarization significantly increased \( \left[ Ca^{2+}\right]_{\text{cyt}} \) and caused mesenteric vasoconstriction.

Mesenteric blood flow (\( Q \)) is decreased when the mesenteric artery constricts and is increased while the vessel relaxes, as shown by the equation \( Q = \Delta P \div MVR \), where \( \Delta P \) is the difference of mean arterial and venous pressure, and MVR is the mesenteric vascular resistance. Because MVR inversely varies as the fourth power of the radius of the artery (MVR = 8\( \eta l/r^4 \), where \( \eta \) is viscosity of blood and \( l \) and \( r \) are the length and radius of the vessels, respectively), the principal determinant of the vascular resistance to blood flow is the caliber of the vessels. Thus a very small decrease in the radius (or diameter) of the mesenteric arterial lumen, due to a modest vasoconstriction, would cause a significant increase in mesenteric vascular resistance and a marked decline in blood flow to the mesenteric capillaries. As shown in this study, blockade of Kv channels in MASMC by 4-AP reduced the diameter (or radius) of the isolated mesenteric arterial rings by \(-20\%\), which would significantly decrease the blood flow through mesenteric arterioles and capillaries and subsequently inhibit the transportation of absorbed nutrients.

![Image](http://jap.physiology.org/10202334) Fig. 11. Schematic diagram showing the possible role of Kv channel function in the regulation of blood flow through mesenteric arteries (MA) and of glucose transport through intestinal epithelial cells. Absorption of digested food from the intestinal lumen into the blood and transportation of the absorbed nutrients via mesenteric circulation to liver and adipose tissues are 2 major processes in energy intake (A). Inhibition of Kv channels with 4-AP caused plasma membrane depolarization, which would inhibit Na\(^+\)-dependent nutrient absorption by reducing the inwardly directed Na\(^+\) driving force in IEC (B) and attenuate nutrient transportation by causing mesenteric vasoconstriction and a decrease in mesenteric blood flow (C). Glu, glucose; ( ), inhibition.

**Possible role of \( E_m \) in the absorption of glucose and amino acids in intestinal epithelial cells.** Absorption of monosaccharides and amino acids from the intestinal lumen into the blood relies on import of the substances into IEC followed by export from the cells into the fluid surrounding the basolateral surface. Glucose and amino acids are mainly imported into IEC by Na\(^+\)-dependent glucose and amino acid symporters located in the apical membrane (36, 42). These symporters are driven by the transmembrane Na\(^+\) concentration gradient and electrical potential difference (\( E_m \)). The negative \( E_m \) in IEC is a major driving force for the movement of Na\(^+\) into the cell under conditions that the transmembrane Na\(^+\) concentration gradient remains unchanged. Thus the membrane depolarization due to blocked Kv channels may also reduce the Na\(^+\) driving force in IEC, attenuate Na\(^+\)-dependent glucose and amino acid uptake into IEC, and subsequently inhibit the absorption of ingested nutrients.

**Possible mechanisms involved in the anorexic effects of fenfluramine and 4-AP.** In addition to inhibiting serotonin transporters (2), the appetite suppressants fenfluramine and dexfenfluramine have been demonstrated to attenuate the mRNA and protein expression of Kv channel \( \alpha \)-subunits (40) and decrease the whole cell \( I_{K(V)} \) (19, 24). 4-AP is a potent blocker of K\(^+\) channels (23, 27, 44); it appears to be more selective to block Kv channels in vascular smooth muscle cells at doses of...
≤5 mM (23, 27). The important roles of Kv channel activity in the regulation of Er in MASMC and IEC direct us to speculate that fenfluramine and 4-AP may similarly exert their anorexigenic effects on rats by blocking K+ channels. The membrane depolarization in MASMC opens VDCC, increases [Ca²⁺]cyt, causes mesenteric vasoconstriction, and inhibits transportation of absorbed nutrients via mesenteric circulation. The membrane depolarization in IEC decreases the transmembrane Na⁺ driving force that is required for the Na⁺-driven uptake of digested nutrients into intestinal epithelial cells and inhibits the absorption of nutrients (Fig. 11). Restriction of nutrient absorption and transportation would significantly contribute to reduce energy intake and weight gain. This study provides a new concept for developing specific blockers of K+ channels in IEC and MASMC as drugs to reduce weight gain.

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