Mechanism of Differential Cardiovascular Response to Propofol in Dahl Salt-Sensitive, Brown Norway, and Chromosome 13-Substituted Consomic Rat Strains: Role of Large Conductance Ca\(^{2+}\) and Voltage-Activated Potassium Channels

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ABSTRACT

Cardiovascular sensitivity to general anesthetics is highly variable among individuals in both human and animal models, but little is known about the genetic determinants of drug response to anesthetics. Recently, we reported that propofol (2,6-diisopropylphenol) causes circulatory instability in Dahl salt-sensitive SS/JRHsdMcwi (SS) rats but not in Brown Norway BN/NHsdMcwi (BN) rats and that these effects are related to genes on chromosome 13. Based on the hypothesis that propofol does target mesenteric circulation, we investigated propofol modulation of mesenteric arterial smooth muscle cells (MASMC) in SS and BN rats. The role of chromosome 13 was tested using SS-13BN/Mcwi and BN-13 SS/Mcwi consomic strains with chromosome 13 substitution. Propofol (5 \(\mu\)M) produced a greater in situ hyperpolarization of MASMC membrane potential in SS than BN rats, and this effect was abrogated by iberiotoxin, a voltage-activated potassium (BK) channel blocker. In inside-out patches, the BK channel number, \(P_o\), and apparent Ca\(^{2+}\) sensitivity, and propofol sensitivity all were significantly greater in MASMC of SS rats. The density of whole-cell BK current was increased by propofol more in SS than BN myocytes. Immunolabeling confirmed higher expression of BK subunit in MASMC of SS rats. Furthermore, the hyperpolarization produced by propofol, the BK channel properties, and propofol sensitivity were modified in MASMC of SS-13BN/Mcwi and BN-13 SS/Mcwi strains toward the values observed in the background SS and BN strains. We conclude that differential function and expression of BK channels, resulting from genetic variation within chromosome 13, contribute to the enhanced propofol sensitivity in SS and BN-13 SS/Mcwi versus BN and SS-13 BN/Mcwi strains.

The sensitivity to general anesthetics is highly variable among strains of rats and individual patients. This is thought to be due in part to genetic differences, but little is known about the pharmacogenetics of the response to anesthetics or the mechanisms involved. We recently identified a major strain difference in the cardiovascular sensitivity to anesthetics whereby Dahl salt-sensitive SS/JRHsdMcwi (SS) rats exhibited a much higher cardiovascular sensitivity to general anesthetic agents than control salt-resistant Brown Norway BN/NHsdMcwi (BN) rats. This was evidenced by the cardiovascular collapse that occurred at lower concentrations of infused pentobarbital (Stekiel et al., 2004, 2006) or propo-
fol (Stekiel et al., 2007) in SS compared with BN. In follow-up studies, using a panel of consomic strains in which individual chromosomes from the BN rat were introgressed one at a time into the homogenous genetic background of the SS rat, and vice versa (Cowley et al., 2001; Roman et al., 2002; Kunert et al., 2006), we determined that introgression of BN chromosome 13 into the genetic background of SS (consomic SS-13BN/Mcwi strain) normalized the propofol sensitivity of SS rats. Conversely, propofol sensitivity of BN rats was elevated to the level typical for SS rats in the consomic BN-13SS/Mcwi strain in which SS chromosome 13 was introgressed into the genetic background of BN rats (T. A. Stekiel, S. J. Contney, A. Stadnicka, C. Moreno, and R. J. Roman, unpublished data).

The major characteristic of the anesthetic-induced cardiovascular collapse is an irreversible drop in the arterial blood pressure, suggesting a significant contribution from the compromised peripheral vascular capacitance and resistance. To this end, general anesthetics such as isoflurane and pento-barbital were reported to cause vasodilation of peripheral resistance vessels, specifically the small mesenteric arteries and veins, in part by hyperpolarizing the membrane potential of vascular smooth muscle cells (Yamazaki et al., 1998, 2002; Kokita et al., 1999; Stekiel et al., 2001; Nagakawa et al., 2003). This effect was attenuated by blockers of the large conductance Ca2+ and voltage-activated potassium (BK) channels (Kokita et al., 1999; Nagakawa et al., 2003).

BK channels are the class/superfamily of potassium channels found in the plasma membrane of the vascular smooth muscle cells that are activated by membrane depolarization and/or by local elevation of cytosolic Ca2+ released via ryanodine-sensitive channels on the smooth muscle sarcoplasmic reticulum. Structurally, the arterial smooth muscle BK channel is composed of four pore-forming α subunits and four accessory β1 subunits, which function to enhance the channel Ca2+ affinity and voltage sensitivity (Toro et al., 1998). The plasma membrane BK channels exist in complexes with voltage-gated calcium channels, ryanodine receptors, protein kinases, and phosphatases and other signaling proteins (Ghatta et al., 2006; Lu et al., 2006). BK channels contribute to regulation of vascular tone by participating in both vasodilation and vasoconstriction (Nelson et al., 1995; Brenner et al., 2000; Alioua et al., 2002; Toro et al., 2004).

The intravenous anesthetic propofol is now commonly used for induction and maintenance of general anesthesia in both outpatient/ambulatory and inpatient surgery because of its highly desirable clinical profile: characteristic rapid onset and emergence and relatively few persistent side effects (White, 2008). However, as is also the case with other general anesthetics, there is great variability in sensitivity to propofol that may lead to circulatory instability among patients and strains of rats (Stekiel et al., 2007).

The present study addressed the mechanisms of differential cardiovascular sensitivity to propofol. We hypothesized that circulatory instability induced by propofol is in part due to its effects on mesenteric circulation, in particular, the small mesenteric arteries, which are important contributors to systemic vascular resistance, and involves hyperpolarization of vascular smooth muscle cells via activation of BK channels. We investigated whether variability in sensitivity to propofol could be attributed to genetic differences in the properties, function, and expression of the BK channel. The availability of reciprocal, consomic strains with chromosome 13 substitution (SS-13BN/Mcwi and BN-13SS/Mcwi) allowed us to directly test whether chromosome 13 plays a role in differential cardiovascular sensitivity to anesthetics, as a first step to identifying the genes involved in this effect.

Materials and Methods

Animals. Experiments were performed on four strains of rats. The two parental strains were the Dahl salt-sensitive (SS/JrHsdMcwi, abbreviated as SS, RGD ID 61499) and the Brown Norway (BN/NHsdMcwi, abbreviated as BN, RGD ID 61498). The two consomic strains with a single homozygous chromosome 13 substitution were SS-13BN/Mcwi (RDG ID 629523) and BN-13SS/Mcwi (RDG ID 2303972) strains. In the SS-13BN/Mcwi strain, chromosome 13 of the BN rat was introgressed onto the homogenous genetic background of the SS rat. In the BN-13SS/Mcwi strain, the reciprocal substitution was created by introgression of chromosome 13 of the SS rat into the genetic background of the BN rat. Consomic strains were obtained from PhysGen (the Program for Genomic Applications at the Medical College of Wisconsin, Milwaukee, WI) and were derived as described previously (Cowley et al., 2004). From birth, all rats were fed a low-salt diet (0.4% NaCl) to minimize the development of hypertension and associated end-organ damage in SS rats. Age-matched male rats (250–400 g; 8–12 weeks old) were used for experiments. All animal protocols were approved by the Institutional Animal Care and Use Committee of the Medical College of Wisconsin.

In Situ Measurement of Mesenteric Arterial Smooth Muscle Membrane Potential. Rats were anesthetized with 2.5% isoflurane (Forane; Baxter, Deerfield, IL) in 30% O2, 70% N2 carrier gas administered via an Ohio Medical Products vaporizer (Airco Inc., Madison, WI). The end tidal CO2 (35–40 mm Hg) and isoflurane concentrations were monitored with POET2 infrared capnograph and end tidal agent monitor (Criticare Systems, Inc., Waunakee, WI). Surgical preparation included tracheotomy and cannulation of femoral artery and femoral vein. A midline laparotomy was performed, and a loop of terminal ileum was exteriorized, placed into a temperature-controlled recording chamber, and superfused with physiologic salt solution containing 119 mM NaCl, 4.7 mM KCl, 1.17 mM MgSO4, 1.6 mM CaCl2, 24 mM NaHCO3, 1.18 mM NaHPO4, and 0.026 mM EDTA, pH 7.4. Physiologic salt solution was maintained at 37°C and was continuously gassed with 90% N2, 5% O2, 5% CO2. A segment of small mesenteric artery (200 μm o.d.) was cleared of perivascular connective tissue and fat without disturbing luminal flow and was stabilized with 50-μm-o.d. stainless steel pins anchored in the silastic base of the recording chamber. Inhaled isoflurane was adjusted to 1% to maintain anesthesia, and animals were allowed to breathe spontaneously. Membrane potential (Em) was measured in situ from the vessel adventitial side with a borosilicate glass microelectrode (FHC Inc., Bowdoinham, ME) filled with 3 M KCl (tip diameter, 0.1 μm; impedance, 40–60 MΩ). Microelectrodes were inserted manually using a hydraulic micromanipulator (Trent Wells Inc., Couterville, CA). Data were recorded using a Grass RSP7C polygraph (Astro-Med/Grass Inc., West Warwick, RI) and SuperScope II, version 1.44 digital data acquisition system (GW Instruments, Somerville, MA). Recordings were made before, during, and after vessel superfusion with 5 μM propofol (International Union of Pure and Applied Chemistry name, 2,6-diisoproplyphenol; Sigma-Aldrich, St. Louis, MO). At least five Em recordings, each of 6 s or greater duration, were averaged to obtain each successive data point under each experimental condition. A similar experimental protocol was followed when investigating the effects of propofol-induced hyperpolarization of Em in the presence of the BK channel blocker ibotenic acid (bTX; 100 nM) or the BK opener NS1819 (5 μM).

Isolation of Mesenteric Arterial Myocytes. Second- and third-order (200–300-μm-o.d.) arteries were dissected from the mesentery of 2.5% isoflurane-anesthetized, age-matched SS, BN, and consomic
SS-13\textsuperscript{BN}/Mcwi and BN-13\textsuperscript{SS}/Mcwi rats. Mesenteric arterial smooth muscle cells (MASMIC) were isolated by the procedure described in Kubo et al. (1997). In brief, after removing perivascular fat, small mesenteric arteries were excised and placed for 15 min into an ice-cold buffer composed of 137 mM NaCl, 5.6 mM KCl, 0.42 mM Na\textsubscript{2}HPO\textsubscript{4}, 0.44 mM NaH\textsubscript{2}PO\textsubscript{4}, 10 mM HEPES, 1 mM MgCl\textsubscript{2}, 1 mM CaCl\textsubscript{2}, and 10 mM glucose, pH 7.3 adjusted with NaOH. The tissue was transferred to the digestion buffer supplemented with 0.1 mM CaCl\textsubscript{2} and containing 1.5 mg/ml papain, 1.0 mg/ml dithioerythritol, and 0.5 mg/ml bovine serum albumin for 30-min incubation at 35°C. This was followed by 20-min incubation at 35°C in the digestion buffer containing 1.5 mg/ml collagenase type F, 1.0 mg/ml hyaluronidase type I-S, and 0.5 mg/ml bovine serum albumin (all from Sigma-Aldrich). At the end of the incubation, the tissue was washed in enzyme-free buffer and triturated with a large-bore transfer pipette to release cells. Dispersed cells were kept at 4°C and were used for patch-clamp experiments within 5 h after isolation.

**Patch-Clamp Experiments.** BK channel currents were monitored in the excised inside-out patch and the whole-cell configurations of the patch-clamp technique (Hamill et al., 1981), using an EPC7 patch-clamp amplifier (ALA Scientific Instruments, Westbury, NY), Digidata 1322A interface (Molecular Devices, Sunnyvale, CA), and an IBM computer running pClamp9 software (Molecular Devices). Borosilicate glass (no filament) pipettes (Garner, Clairmount, CA) had resistances of 6 to 9 MΩ and 2 to 5 MΩ for single-channel and whole-cell recordings, respectively.

The pipette/extracellular solution for single-channel recordings consisted of 145 mM KCl, 0.5 mM MgCl\textsubscript{2}, 0.5 mM CaCl\textsubscript{2}, and 10 mM HEPES, pH 7.4. The bath/intracellular solution at pH 7.3 consisted of 145 mM KCl, 0.5 mM MgCl\textsubscript{2}, 10 mM HEPES, 2 mM EGTA, and CaCl\textsubscript{2} added at concentrations yielding 0.1, 1.0, or 10 µM free intracellular Ca\textsuperscript{2+} as calculated with WinMAXC32 software (C. Patton, Stanford University, Pacific Grove, CA). Single-channel currents were filtered at 500 Hz (eight-pole Bessel filter) and recorded at a 5-µs sampling interval in a gap-free mode at the membrane potential of +40 mV. The unitary current amplitude, conductance, and $P_p$ were determined from all-points histograms using pClamp9 software (Molecular Devices) and Origin7 (OriginLab Corp., Northampton, MA). The number of BK channels per patch, unitary current amplitude, single-channel conductance, and $P_p$ were determined from the amplitude histograms. The mean $P_p$ was calculated by the binomial distribution $[1 - (P_p)]^{n}$, where $n$ is the number of active channels in the patch and $P_p$ is the channel closed probability derived from the Gaussian fits to the amplitude histograms. In single-channel analysis, the detection threshold for opening and closing was set using a half-amplitude criterion. The number of BK channels per patch was estimated under conditions of maximal channel opening at the membrane potential of +80 mV and in the presence of 10 µM free intracellular Ca\textsuperscript{2+} (Nelson et al., 1995; Meera et al., 1996).

The extracellular solution for whole-cell outward K\textsuperscript{+} current recordings was composed of 130 mM NaCl, 5 mM KCl, 1 mM MgCl\textsubscript{2}, 2 mM CaCl\textsubscript{2}, 10 mM HEPES, and 10 mM glucose, pH 7.4. The pipette solution for whole-cell recordings at the holding potential ($V_h$) of −60 mV contained 130 mM K-aspartate, 30 mM KCl, 10 mM NaCl, 1 mM MgCl\textsubscript{2}, 0.5 mM EGTA, and 10 mM HEPES, pH 7.3. The pipette solution for recordings at $V_h$ of −35 mV was composed of 150 mM KCl, 5 mM NaCl, 1.0 mM MgCl\textsubscript{2}, 10 mM HEPES, 2 mM EGTA, and CaCl\textsubscript{2} that was added from 1 M stock in the amounts (calculated with WinMAXC32; see above) yielding 0.1 and 1 µM free intracellular Ca\textsuperscript{2+}. The pH was 7.2. Whole-cell K\textsuperscript{+} currents were evoked by 300-ms voltage steps from $V_h$ of −60 or −35 mV to potentials ranging from −60 to +80 or +60 mV in 20-mV increments. Recordings were filtered at 1 kHz, digitized, and stored for offline analysis. Identities of single BK channels was confirmed by blockade with 100 nM penitrem A (tremorin A, C\textsubscript{37}H\textsubscript{44}CINO\textsubscript{6}) and 1 µM paxilline (C\textsubscript{27}H\textsubscript{33}NO\textsubscript{4}). Whole-cell currents were blocked with 1 µM paxilline or 300 nM IbTX. All experiments were performed at room temperature (20–24°C). The 100 mM stock of propofol in dimethyl sulfoxide was diluted in recording buffer. Dimethyl sulfoxide alone had no effect on BK channel activity. All standard chemicals and iberiotoxin, paxilline, penitrem A, and NS1619 were purchased from Sigma-Aldrich.

**Immunohistochemistry.** Small mesenteric arteries were obtained from isoflurane-anesthetized parental SS and BN rats for immunolabeling of BK channel proteins. Freshly dissected vessels were washed in ice-cold 0.1 M phosphate-buffered saline (PBS) and slit open longitudinally. The vessels were fixed for 10 min in suspension in 1% paraformaldehyde, permeabilized with 0.5% Triton X-100 in PBS, and rinsed with PBS. Thereafter, the vessels were incubated for 1 h at 37°C with the rabbit polyclonal primary antibodies for BK channel α subunit (anti-KCa\textsuperscript{2+}1.1/ KCNMA1; Alomone Labs, Jerusalem, Israel; 1:50 dilution in PBS) and β\textsubscript{i} subunit (anti-slo1/ KCNMBl; Alomone Labs; 1:100 dilution in PBS). Antibodies preincubated with the control antigens were used as negative controls. After incubation with primary antibodies, the vessels were washed three times for 5 min each with PBS, incubated 30 min at 37°C with Alexa Fluor 488-conjugated goat anti-rabbit IgG secondary antibody (Invitrogen/Molecular Probes, Carlsbad, CA) at a 1:1000 dilution in PBS, and washed in PBS. Nuclear staining was accomplished with 3-min exposure to 1 µM TO-PRO-3 (Invitrogen/Molecular Probes) at room temperature. After a final rinse in PBS, the vessels were mounted on positively charged Colorfrost Plus microscope slides (Thermo Fisher Scientific, Waltham, MA). Immunofluorescence was measured in 10 randomly selected visual fields from each vessel. Images were captured at 400× magnification, under fluorescence confocal laser-scanning microscope (Nikon, Tokyo, Japan) using a krypton/argon laser imaging system. Fluorescence excitation and emission were set at 488 and 520 nm, respectively, for Alexa Fluor 488 and at 633 and 661 nm, respectively, for TO-PRO-3. Data were analyzed with MetaMorph software (Universal Imaging Corporation). Results are expressed as averaged fluorescence intensity.

**Statistical Analysis.** Mean ± S.E.M. are presented. Statistical analysis was performed using the analysis of variance with a post hoc Bonferroni/Dunn analysis, and $n$ indicates the number of rats used in each experimental group. Differences were accepted as significant at $p < 0.05$.

**Results.**

**Propofol Effects on the in Situ $E_m$ of Small Mesenteric Arterial Smooth Muscle in SS, BN, SS-13\textsuperscript{BN}/Mcwi, and BN-13\textsuperscript{SS}/Mcwi Rats.** To test whether differences in cardiovascular sensitivity to propofol are due in part to strain-related genetic differences on chromosome 13, we compared $E_m$ measured in situ in the intact small mesenteric arteries of four strains of rats, i.e., parental SS and BN rats and consomic SS-13\textsuperscript{BN}/Mcwi and BN-13\textsuperscript{SS}/Mcwi strains ($n = 10$/group) before, during, and after superfusion of the vessels with 5 µM propofol. As shown in Fig. 1a, despite a similar baseline $E_m$ (average, −36.6 ± 0.2 mV), propofol caused a differential hyperpolarization of $E_m$ in four strains of rats. The $\Delta E_m$ (Fig. 1b) produced by propofol was significantly greater in parental SS rats (10.5 ± 0.5 mV) and consomic BN-13\textsuperscript{SS}/Mcwi strain (11.0 ± 0.3 mV) than in parental BN (2.3 ± 0.4 mV) and the consomic SS-13\textsuperscript{BN}/Mcwi strain (2.8 ± 0.3 mV) that carry chromosome 13 of BN rats. This suggested that strain differences in $E_m$ hyperpolarization by propofol are related to genes located on chromosome 13.

**Effects of IbTX and NS1619 on the in Situ $E_m$ of Small Mesenteric Arterial Smooth Muscle in SS, BN, SS-13\textsuperscript{BN}/Mcwi, and BN-13\textsuperscript{SS}/Mcwi Rats.** Because BK channel current is the major determinant of membrane potential of vascular smooth muscle cell, we investigated whether hyperpolarization of $E_m$ by propofol is due in part to activation of...
BK channel in the mesenteric arteries. In SS and BN rats (n = 6/group), \( E_m \) was measured in situ before and during vessel superfusion with 100 nM IbTX, the blocker of BK, followed by superfusion with 100 nM IbTX and 5 μM propofol. Figure 2 shows that IbTX alone depolarized \( E_m \) in SS (10.6 ± 0.8 mV) and BN (9.4 ± 0.8 mV) rats by a similar extent. Furthermore, in the presence of IbTX, propofol failed to hyperpolarize \( E_m \) in vessels of either SS or BN rats, indicating the effects of propofol actions involves activation of BK channel.

With a focus on chromosome 13, \( E_m \) was measured in situ in small mesenteric arteries of SS, BN, SS-13BN/Mcwi, and BN-13SS/Mcwi rats (n = 6/group) before and during vessel superfusion with the opener of BK channel, 5 μM NS1619, followed by NS1619 plus 5 μM propofol. As shown in Fig. 3, a and b, NS1619 caused \( E_m \) hyperpolarization in four strains. This effect was significantly greater in parental SS and consomic BN-13SS/Mcwi strains than in parental BN and consomic SS-13BN/Mcwi strains. Together, data from the in situ experiments demonstrated that propofol causes differential hyperpolarization of mesenteric arterial smooth muscle through activation of BK channels and that the differences between strains are related to genes located on chromosome 13.

Properties of BK Channels in MASMC of SS, BN, SS-13BN/Mcwi, and BN-13SS/Mcwi Rats. Because the in situ experiments suggested BK channel involvement and apparent strain differences in hyperpolarization produced by propofol, we compared the properties of single BK channels...
in MASMC of four strains by patch-clamp methodology. Figure 4a shows representative recordings of single BK channel activity in excised inside-out membrane patches from MASMC of SS, BN, SS-13BN, and BN-13SS rats at +40-mV patch potential and in the presence of three physiologically relevant concentrations of intracellular free Ca$^{2+}$. The probability of BK channel opening was greater in MASMC of SS rats than BN rats. Furthermore, channel activity was influenced by substitution of chromosome 13 in that the $P_o$ of SS-13BN/Mcwi channels was low and similar to that of BN channels; and conversely, the $P_o$ of channels in BN-13SS/Mcwi rats was similar to that of SS rats.

Figure 4b summarizes data for mean $P_o$ determined at 0.1, 1.0, and 10 μM free intracellular Ca$^{2+}$ and shows that calcium sensitivity of BK channels of parental BN rats ($n = 13$) was significantly lower than that of parental SS rats ($n = 10$) at all tested concentrations of free intracellular Ca$^{2+}$. Furthermore, the Ca$^{2+}$ sensitivity of channels in SS-13BN/Mcwi rats ($n = 10$) was similar to that of BN rats, whereas the Ca$^{2+}$ sensitivity of BN-13SS/Mcwi rats ($n = 10$) resembled that of SS rats. This implies that chromosome 13 carries a gene or genes encoding for a modulator of the Ca$^{2+}$ sensitivity of the BK channel.

Because the differences in the channel conductance, or channel density in the plasma membrane, also may account for $P_o$ differences between SS and BN rats, the unitary conductance was measured and the number of BK channels per patch was determined under conditions of maximal channel opening, i.e., at +80-mV membrane potential and in the presence of 10 μM free intracellular Ca$^{2+}$. The conductance of single BK channels was similar in SS rats ($273 \pm 10$ pS; $n = 20$) and BN rats ($275 \pm 11$ pS; $n = 20$). However, the number of BK channels/patch was significantly lower in BN rats (5 ± 2 channels; $n = 12$), SS-13BN/Mcwi rats (4 ± 1 channels; $n = 10$), and BN-13SS/Mcwi rats (4 ± 1 channels; $n = 9$) compared with channel number seen in SS rats (9 ± 1 channels; $n = 10$).

**Effects of Propofol on BK Channel in Inside-Out Patches.** To test whether propofol directly activates the BK channel, $P_o$ was measured before (control) and during 5 μM propofol application to the cytosolic side of excised membrane patches. Recordings were made at ±40 mV in the presence of 1 μM free intracellular Ca$^{2+}$. Figure 5a presents the mean $P_o$ determined for BK channels of four strains in the absence and presence of propofol. Figure 5b summarizes the effects of propofol on BK channel, showing that propofol increased $P_o$ in SS rats ($33 \pm 19\%$; $n = 7$) and BN-13SS/Mcwi rats ($27 \pm 12\%$; $n = 5$) but not in BN rats ($14 \pm 20\%$; $n = 10$) or SS-13BN/Mcwi rats ($16 \pm 10\%$; $n = 5$). Thus, $P_o$ of BK channels in MASMC is differentially modulated by propofol even under in vitro conditions, and the observed differences between SS and BN rats seem related to a gene or genes on chromosome 13.

**Effects of Propofol on Whole-Cell Outward K$^+$ Current.** The effects of propofol on the outward K$^+$ current in MASMC were examined under whole-cell voltage clamp at two different $V_h$ (−60 and −35 mV) to determine whether this anesthetic affects BK channel activity when applied to the outside of the cell. In first set of experiments, outward K$^+$ currents were elicited by test pulses ranging from −60 to +80
mV in 20-mV increments, from \( V_h \) of \(-60 \) mV. Representative traces of current recorded using such protocol are shown in Fig. 6a. Under these conditions, blockade of BK channels with 300 nM IbTX or 100 nM penitrem A reduced outward currents only by approximately 40%, suggesting other K⁺ channels contribute to outward K⁺ conductance at \( V_h \) of \(-60 \) mV. The current-voltage curves in Fig. 6b show that at depolarized membrane potentials from +20 to +80 mV basal current density (current normalized to cell capacitance) was approximately 2-fold higher in parental SS rats than in BN rats. When measured during a voltage step to +80 mV from \( V_h \) of \(-60 \) mV, current density was \( 38 \pm 4.8 \) pA/pF in SS rats (\( n = 7 \)) and \( 18 \pm 2.2 \) pA/pF in BN rats (\( n = 7 \)). Cell superfusion with 5 \( \mu \)M propofol caused a small increase in current density in SS rats (\( n = 7 \)) but not in BN (\( n = 7 \)), SS-13\(^{BN} \)/Mcwi (\( n = 5 \)), or BN-13\(^{SS} \)/Mcwi (\( n = 5 \)) myocytes (Fig. 6c).

In second set of experiments, the effects of propofol on whole-cell outward K⁺ currents were examined in MASMC of parental SS and BN rats under conditions of tightly controlled free intracellular Ca²⁺ (0.1 and 1.0 \( \mu \)M) and at more physiological membrane potential of \(-35 \) mV. Figure 7 shows that the outward K⁺ currents elicited by test pulses from \(-60 \) mV to +60 mV from \( V_h \) of \(-35 \) mV (Fig. 7a) increased during cell superfusion with 5 \( \mu \)M propofol (Fig. 7b), and the current was completely blocked (within 5 min) by 1 \( \mu \)M paxilline (Fig. 7b), suggesting that the outward K⁺ current measured at \(-35 \) mV \( V_h \) is primarily determined by BK channels. In Fig. 7c, example traces of currents recorded from MASMC of SS and BN rats at 1.0 \( \mu \)M free intracellular Ca²⁺ before and during cell superfusion with 5 \( \mu \)M propofol demonstrate differences in the basal current levels and propofol sensitivity between SS and BN rats. Figure 7d summarizes the effects of 5 \( \mu \)M propofol on density of whole-cell outward current (IBK) at 0.1 and 1.0 \( \mu \)M free intracellular Ca²⁺ measured during voltage steps from \(-35 \) to +20, +40, and +60 mV in MASMC of SS rats (\( n = 3 \)) and BN rats (\( n = 3 \)). Propofol data are expressed as percentage of control current density. The results demonstrate strain dependence and intracellular free Ca²⁺ dependence of the effects of propofol on IBK. The voltage dependence is evident in all but the SS rats at 0.1 \( \mu \)M free Ca²⁺ group.
Propofol increases whole-cell BK current (IBK) in MASMC of SS and BN rats at −35 mV $V_h$. Whole-cell $K^+$ currents were recorded in MASMC at K⁺ gradient of 5 mM K⁺ outside and 150 mM K⁺ inside and at 0.1 or 1.0 μM free intracellular Ca²⁺. a, cells were held at −35 mV, and currents were elicited during 300-ms voltage steps from −60 to +60 mV in 20-mV increments. b, traces of currents recorded at 1.0 μM internal free Ca²⁺ from BN myocyte in control, during application of 5 μM propofol plus 1 μM paxilline. Paxilline blocked control and propofol-induced current. c, example traces of IBK recorded from SS myocyte and BN myocyte in the absence (control) and presence of 1 μM propofol. d, summary of propofol effects on IBK in SS and BN myocytes at 0.1 or 1.0 μM intracellular free Ca²⁺. Propofol data are expressed as percentage of control current density measured during voltage steps to +20, +40, and +60 mV from −35 mV $V_h$. * p < 0.05, individual groups versus respective controls; #, p < 0.05 BN versus corresponding SS group at 0.1 or 1.0 μM intracellular free Ca²⁺.

Expression of BK Channel Subunit in Small Mesenteric Arteries of SS and BN Rats. Figure 8a shows images of immunolabeled BK α subunit in MASMC of SS (left) and BN (right) vessels. The plasma membrane and cytosolic fluorescence intensity was greater in MASMC of SS small mesenteric arteries. Figure 8b summarizes the densitometry measurements/data for BK channel α subunit and β1 subunit, showing that expression of α but not β1 subunit was significantly greater in MASMC of SS rats compared with BN rats (n = 3/group).

Discussion

The present study addressed the underlying mechanisms of differential cardiovascular sensitivity to propofol in SS and BN rats (Stekiel et al., 2007). The major findings of this work are as follows: 1) in situ, propofol causes greater hyperpolarization of mesenteric arterial smooth muscle $E_{p}$ in parental SS rats than in BN rats, and this effect is related to genes on chromosome 13; 2) propofol-induced hyperpolarization is prevented by IbTX, indicating involvement/activation of BK channels; and 3) the properties of BK channels are different in MASMC of parental SS and BN rats. The channel $P_o$, and apparent $K^+$ sensitivity, in particular, are greater in SS rats than in BN rats, and this difference could be related to gene(s) on chromosome 13. Furthermore, the number of channels per patch and the BK α subunit expression also are greater in SS rats. 4) Propofol increases the $P_o$ of BK channel in MASMC of SS rats but not BN rats, and the difference is associated with SS chromosome 13. In addition, propofol enhances the whole-cell IBK in MASMC of SS rats more than in BN rats. 5) The expression of the BK channel α subunit is significantly higher in mesenteric arteries of SS rats compared with BN rats.

Propofol, a modern intravenous general anesthetic, is routinely used for induction and maintenance of general anesthesia because of rapid onset and emergence and relatively few side effects. Yet, cardiovascular sensitivity to propofol shows marked variability among patients, probably due to genetic differences. In humans, both children and adults, under circumstances such as prolonged infusions for the purpose of long-term sedation, propofol may cause cardiovascu-
lar collapse (Bray, 2002). The mechanisms of propofol actions are thought to involve modulation of the central sympathetic control (Krassiovikov et al., 1993), inhibition of the local vascular sympathetic activity (Pensado et al., 1993), and direct modulation of the vascular smooth muscle. Propofol can induce hypotension by lowering peripheral vascular resistance; and in humans and animals exhibiting exaggerated anesthetic sensitivity, propofol-induced hypotension can be severe. In various peripheral vascular beds, including the mesenteric bed, propofol has complex endothelium-dependent and endothelium-independent effects that lead to a decrease in vascular smooth muscle tone (Nakamura et al., 1992; Boillot et al., 1999; Yamashita et al., 1999; Yamazaki et al., 2002; Nagakawa et al., 2003). A recent pharmacological study on isolated coronary arteries suggested that propofol-induced vasodilation is mediated by activation of BK channels (Klockgether-Radke et al., 2004). Our study corroborates this hypothesis by showing that propofol directly modulates activity of BK channels in MASMC. However, compared with the pronouneled hypertpolarization of mesenteric vesels smooth muscle, the effects of propofol on $P_o$ of BK channel in inside-out patches and density of whole-cell outward K+ current are relatively modest. One explanation could be a difference in experimental design. The in situ experiments were performed at 37°C in small mesenteric arteries with intact blood flow and intact innervation, whereas patch-clamp experiments were performed at 20–24°C in isolated myocytes or excised membrane patches. Thus, the in situ findings could imply a possibility of existence of other propofol targets, for example, a neural component that is absent in isolated myocytes and membrane patches. Furthermore, we cannot ignore that despite their high plasma membrane expression, the activity of potassium channels in the arterial smooth muscle cells is relatively low under normal physiologic conditions. However, because of high-input resistance of the plasma membrane, the opening or closing of even very few BK channels that have high conductance can still lead to substantial changes in the membrane potential (Standen and Quayle, 1998). Thus, although the direct effects of propofol on BK channel activity are relatively modest, they can potentially lead to a greater membrane hyperpolarization in SS rats, such as is observed in situ.

The results of the present study indicate that propofol increases $P_o$ of BK channels in SS rats and, in MASMC of both SS and BN rats, it enhances whole-cell IBK at physiological membrane potential of ~35 mV. Compared with BN rats, the greater effects of propofol in SS rats correlate well with higher expression of BK channels in the membrane of MASMC, greater BK channel $P_o$ and Ca2+ sensitivity, and greater whole-cell IBK density. This fits with previous observations that the expression of BK channels is elevated in the vascular smooth muscle of various circulatory beds in spontaneously hypertensive rats (Cox et al., 2001; Cox, 2002; Cox and Rusch, 2002; Chang et al., 2006). In SS rats, this could be a part of a physiological compensatory mechanism to slow the development of hypertension when on a low-salt diet (Rapp, 1994; Garrett et al., 2003). Fed a high-salt diet, SS rats rapidly develop a low renin form of hypertension, but they remain normotensive over the age range used in our study when on a low-salt diet (Rapp, 1994; Cowley et al., 2001; Kunert et al., 2006). However, even under such conditions the properties of BK channel in MASMC of SS rats are significantly different from those of BN rats. One explanation could be an impaired renin-angiotensin system in SS rats. Compared with BN rats that carry a normal renin gene on chromosome 13, renin activity and angiotensin II (Ang II) levels tend to be reduced in SS rats relative to BN and other strains (Cowley et al., 2001; Drenjancevic-Peric and Lombard, 2005). Low levels of circulating Ang II could potentially be responsible for the enhanced activity of mesenteric BK channel in SS rats. A powerful vasoconstrictor, Ang II modulates activity of BK channels in the vascular smooth muscle cells. This occurs via a mechanism that involves the AT1 receptor-mediated activation of the cytosolic tyrosine kinase c-Src, which phosphorylates the BK channel and depresses its activity (Alioua et al., 2002; Toro et al., 2004). Thus, modulation by Ang II may serve as protective mechanism against excessive hyperpolarization of MASMC. In SS rats that exhibit chronic low levels of circulating Ang II, this compensatory mechanism might be less effective, resulting in the increased activity of BK. In addition, higher channel density and higher plasma membrane expression of BK α subunit in MASMC of SS rats suggests that regulation of channel turnover and trafficking may be different between these strains.

The present study shows that chromosome 13 substitution changes the phenotype of SS-13BN/Mcwi and BN-13SS/Mcwi strains toward that of BN and SS rats, respectively, supporting the notion that the genes on chromosome 13 play a role in the BK channel-mediated mechanism of anesthetic sensitivity. It also suggests that chromosome 13 may carry a gene or genes coding for a modulator of Ca2+ sensitivity of BK channel. However, such gene has not been identified yet. The genes coding for α and β subunit of the vascular BK channel are of less interest here because they are not found on rat chromosome 13. Rather, in the rat, the rslo or KCNMA1 gene coding for BK α subunit and KCNMB1 gene coding for accessory sloβ1 subunit are located on chromosome 15 (15p16) and chromosome 10 (10q12), respectively (Rat Genome Database and GenBank Database).

In conclusion, we propose that the mechanism of cardiovascular actions of propofol involves in part hyperpolarization of MASMC via activation of the BK channel. The parental strain differences in BK channel properties and expression seem to underlie differential sensitivity to propofol, suggesting that background genetic differences cause this effect. Chromosome 13 substitution alters the phenotypic profile of SS-13BN/Mcwi and BN-13SS/Mcwi MASMC strains such that they resemble the opposite parental strains. This supports the concept that chromosome 13-associated genes are responsible for differential cardiovascular sensitivity to propofol.

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References


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