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ORIGINAL ARTICLE

The G protein–coupled estrogen receptor agonist, G-1, attenuates BK channel activation in cerebral arterial smooth muscle cells

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Abstract

The G protein–coupled estrogen receptor (GPER) is a significant modulator of arterial contractility and blood flow. The GPER-specific activator, G-1, has been widely used to characterize GPER function in a variety of tissue types. Large conductance, calcium (Ca^{2+})-activated K^+ (BK) channels are sensitive to 17β-estradiol (17β-E2) and estrogenic compounds (e.g., tamoxifen, ICI 182 780) that target estrogen receptors. The purpose of this study was to investigate the effects of G-1 on BK channel activation and function in cerebral arterial myocytes. Inside-out and perforated patch clamp were utilized to assess the effects of G-1 (50 nmol·L^{-1}-5 μmol·L^{-1}) on BK channel activation and currents in cerebral arterial myocytes. Pressurized artery myography was used to investigate the effects of G-1 on vasodilatory response and BK channel function of cerebral resistance size arteries. G-1 reduced BK channel activation in cerebral arterial myocytes through elevations in BK channel mean close times. Depressed BK channel activation following G-1 application resulted in attenuated physiological BK currents (transient BK currents). G-1 elicited vasodilation, but reduced BK channel function, in pressurized, endothelium-denuded cerebral arteries. These data suggest that G-1 directly suppresses BK channel activation and currents in cerebral arterial myocytes, BK channels being critically important in the regulation of myocyte membrane potential and arterial contractility. Thus, GPER-mediated vasodilation using G-1 to activate the receptor may underestimate the physiological function and relevance of GPER in the cardiovascular system.

KEYWORDS
BK channel, BKca channel, estrogen receptor, G-1, GPER1, GPR30

1 | INTRODUCTION

The G protein–coupled estrogen receptor (GPER, GPR30) is an estrogen-sensitive G protein–coupled receptor (GPCR) that is structurally unique from other estrogen receptors (ERs).[1] First identified in ER-positive breast cancer cell lines and exhibiting a high (~1-

Abbreviations: $[Ca^{2+}]_i$, intracellular calcium; 17β-E2, 17β-estradiol; BK, large-conductance calcium (Ca^{2+})-activated potassium channel; ER, estrogen receptor; G-1, 1-[4-(6-bromobenzo[1,3]dioxol-5-yl)-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinolin-8-yl]-ethanone; GPER, G protein–coupled receptor 30; IBTX, iberiotoxin; PKA, protein kinase A; RyR, ryanodine receptor.

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10 nmol·L^{-1}) affinity for 17β-estradiol (17β-E2), GPER activation leads to a broad range of rapid physiological processes that include protein kinase activation (e.g., Erk-1, Erk-2, and PKA) and intracellular calcium ([Ca^{2+}]$_i$) mobilization.[2-4] In arteries, GPER activation induces vasodilation, which has been reported to involve the generation of cAMP and PKA activation and the activation of potassium (K^+) channels.[5-9]

In arterial smooth muscle cells (myocytes), the classical nuclear ERs (ERα, ERβ) along with GPER are expressed,[10] thus in the characterization of GPER it is important to utilize appropriate pharmacological treatments that specifically target the receptor. G-1 (1-[4-(6-bromobenzo[1,3]dioxol-5-yl)-3a,4,5,9b-tetrahydro-3H-cyclopenta[c].

quinolin-8-yl]-ethanone) was introduced as a specific GPER agonist that exhibited a high affinity (~10 nmol·L^{-1}) for GPER with no appreciable affinity for ERα or ERβ.[11] G-1 administration elicits vasodilation in many different artery types,[7] however, the maximum vasodilatory effect is less than what is achieved with 17β-E2.[12,13] As such, a moderate vasodilatory response is commonly associated with GPER activation.

Large conductance, calcium (Ca^{2+})-activated K^+ (BK) channel activation is a putative downstream mechanism associated with GPER activation.[9] In cerebral arteries, BK channels are critical for the regulation of myocyte plasma membrane potential where channel activation leads to K^+ efflux, membrane hyperpolarization, and vasodilation.[14,15] BK channel function is primarily regulated through Ca^{2+} spark activity, Ca^{2+} release events that occur via ryanodine-sensitive Ca^{2+} (RyR) channels at the sarcoplasmic reticulum.[16,17] Ca^{2+} sparks (~10-30 μmol·L^{-1}) can activate clusters of proximal BK channels on the plasma membrane, which lead to large, transient BK currents.[18,19] BK channels are especially relevant to 17β-E2-mediated vasodilation as 17β-E2 can directly activate BK channels via an extracellular binding site on the regulatory β1 subunit.[20] Subsequent studies of the broad ER (α,β) inhibitor, ICI 182 780, and the selective estrogen receptor modulator, tamoxifen, have also demonstrated that BK channels can be directly modulated by estrogenic compounds leading to alterations in BK channel activation.[21,22] In coronary arterial myocytes, G-1-mediated GPER activation increased BK channel activation leading to vasodilation, which was believed to be independent of direct interactions between G-1 and the BK channel.[9] However, this study did not utilize physiological conditions typically associated with BK channel activation. Given the history of ER-targeting compounds to possess direct effects on BK channel activation, it is important to further assess for direct effects of G-1 on BK channel function. Moreover, a careful study is warranted as much of the vasodilatory capacity attributed to GPER is primarily examined using G-1 as the means to activate the receptor.

2 | MATERIALS AND METHODS

The animal protocol was reviewed and approved by The Florida State University Animal Care and Use Committee and adhered to the guidelines set forth in the Guide for the Care and Use of Laboratory Animals and the ARRIVE guidelines.[23] Female CD (Sprague-Dawley) IGS rats (2 months old) were purchased from Charles River and housed at the Biomedical Research Facility vivarium (22°C, 12:12 hour light-dark cycle, 2-3 rats per cage). Rats received water and standard rat chow ad libitum. On the day of experimentation, rats were euthanized with an intraperitoneal injection of sodium pentobarbital (150 mg·kg^{-1}). The brain was removed and placed in an ice-cold (4°C), HEPES-buffered physiological saline solution (PSS) consisting of (in mmol·L^{-1}): 10 HEPES, 10 glucose, 6 KCl, 134 $NaCl_2$, 2 $CaCl_2$, and 1 $MgCl_2$ (pH 7.4). Resistance size arteries were carefully dissected away from the brain and the connective tissue removed.

2.1 | Cell dissociation

All enzymes were dissolved in Ca^{2+}-free HEPES-buffered PSS that contained (in mmol·L^{-1}): 10 HEPES, 10 glucose, 5.6 KCl, 55 NaCl, 80 Na glutamate, and 2 $MgCl_2$ (pH 7.4). Posterior cerebral and superior cerebellar arteries were placed in Ca^{2+}-free HEPES with 0.56 mg·mL^{-1} papain, 0.8 mg·mL^{-1} 1,4-dithioerythritol, and 1 mg·mL^{-1} bovine serum albumin (BSA) for 11.5 minutes at 37°C. Arteries were then transferred to Ca^{2+}-free HEPES with 0.6 mg·mL^{-1} collagenase type F, 0.3 mg·mL^{-1} collagenase type H, and 1 mg·mL^{-1} BSA for 7 minutes at 37°C. Arteries were washed 2 times with ice-cold Ca^{2+}-free HEPES and triturated with a glass Pasteur pipette to yield individual arterial myocytes. Myocytes were kept on ice until use.

2.2 | Patch-clamp electrophysiology

An Axopatch 200B amplifier and Clampex software (10.6; Molecular Devices, San Jose, CA, USA) were used to record BK currents. For inside-out patch clamp, symmetrical pipette and bath solutions contained (in mmol·L^{-1}): 140 KCl, 10 HEPES, 5 EGTA, 1.6 HEDTA, 2.28 $MgCl_2$, and 5.22 $CaCl_2$ (pH 7.2). Free Ca^{2+} and Mg^{2+} were calculated to be 10 μmol·L^{-1} and 1 mmol·L^{-1}, respectively, using WEBMAXC Standard (http://www.stanford.edu/~cpatton/webmaxcS.htm). Pipette solution for perforated whole-cell patch clamp contained (in mmol·L^{-1}): 10 HEPES, 30 KCl, 10 NaCl, 1 $MgCl_2$, 0.05 EGTA, and 110 K aspartate (pH 7.2). A total volume of 2 μL of amphotericin B solution (amphotericin B dissolved in DMSO [1 mg·15 μL^{-1}]) was added to 500 μL of pipette solution (~267 μg·mL^{-1} amphotericin B) to perforate the plasma membrane for whole cell recording. Bath solution for perforated whole-cell patch-clamp contained (in mmol·L^{-1}): 10 HEPES, 10 glucose, 6 KCl, 134 $NaCl_2$, 2 $CaCl_2$, and 1 $MgCl_2$ (pH 7.4). G-1 was initially dissolved in DMSO and further diluted 1:10 in 100% EtOH. Vehicle (0.45% EtOH, 0.05% DMSO) control experiments were performed to test for vehicle effects on single BK channel activity and patch/cell viability. For inside-out patch-clamp protocols, BK channel activity was allowed to stabilize for 2-3 minutes prior to adding G-1. G-1 was perfused into the bath using gravity flow followed by an additional 2-3 minutes of gap-free recording to detect a G-1 effect. For perforated whole-cell patch-clamp protocols, recording began ~10 minutes after obtaining a giga-ohm seal to allow for perforation of the plasma membrane. Baseline

transient BK currents were recorded for ~5 minutes followed by administration of G-1 as described above. BK single-channel activity (NP_o) was calculated using the following equation: $NP_o = \Sigma\ (t_1 + t_2 \ldots t_i)$, where t_i is the relative open time (time open/total time) for each channel level. Open probability (P_o) was calculated by dividing NP_o by the total number of channels. A transient BK current was defined as a current that exceeded 10 pA at a membrane potential of −40 mV. BK currents were acquired at 5 kHz and filtered at 1 kHz. A Hum Bug Noise Eliminator (Quest Scientific, North Vancouver, MC, Canada) was used to reduced 60 Hz noise during inside-out (single channel) patch clamp. All analyses were performed offline using Clampfit software (10.6, Molecular Devices).

2.3 | Pressurized artery myography

Middle cerebral artery segments were placed in a vessel chamber with MOPS-buffered PSS that contained (in $mmol{\cdot}L^{-1}$): 145 NaCl, 4.7 KCl, 2 $CaCl_2$, 1.17 $MgSO_4$, 1.2 NaH_2PO_4, 5 glucose, 2 pyruvate, 0.02 EDTA, 3 MOPS, and 1 $g{\cdot}100\ mL^{-1}$ bovine serum albumin (pH 7.4). Artery segments were cannulated at each end, secured with nylon suture (Alcon, 11-0 nylon microfilament), and the chamber placed on an inverted microscope (Olympus IX70, Leeds Instruments, Minneapolis, MN, USA). Artery segments were pressurized with MOPS-buffered PSS to ~60 $mm{\cdot}Hg^{-1}$ (~82 $cm{\cdot}H_2O^{-1}$) using hydrostatic columns. Endothelium was rendered nonviable (denuded) by passing 5-8 mL^{-1} of air through the lumen of the artery and tested for absence of vasodilation in response to acetylcholine (10 $\mu mol{\cdot}L^{-1}$). The bath temperature was maintained at 37°C. Intraluminal diameter was measured using video calipers (Colorado Video; Boulder, CO, USA). Arteries equilibrated for ~20-30 minutes to establish myogenic tone before beginning pharmacological experiments. Myogenic tone was calculated as: $100 \times (1 - D_{Act}/D_{Pas})$, where D_{Act} is the active tone diameter and D_{Pas} is maximal diameter achieved with Ca^{2+}-free MOPS-buffered PSS. All drugs for myography were initially dissolved in DMSO and further diluted 1:10 in 100% EtOH. Vehicle in bath was ~0.45% EtOH and ~0.05% DMSO.

2.4 | Statistics

Data and statistical analysis comply with recommendations on experimental design and analysis in pharmacology.[24] Data are expressed as mean ± SE. Statistically significant differences between mean data were identified using a repeated measures analysis of variance (ANOVA). In our experiments, group sizes of 6-8 per group possess sufficient power (>0.80) to detect statistically significant mean differences at $\alpha = 0.05$.

2.5 | Materials

All materials used in this manuscript were purchased from Sigma-Aldrich (St. Louis, MO, USA) with the following exceptions: G-1 (Cayman; Ann Arbor, MI, USA) and iberiotoxin (Bachem; Torrance, CA, USA).

3 | RESULTS

Inside-out patch clamp was performed on freshly isolated cerebral arterial myocytes to determine the effects of G-1 on BK channel activation. Membrane patches were voltage clamped at −40 mV and free Ca^{2+} was maintained at 10 $\mu mol{\cdot}L^{-1}$, parameters that fall within the physiological ranges of cerebral arterial myocytes.[18,25] G-1 (5 $\mu mol{\cdot}L^{-1}$) elicited a reduction in open probability (P_o) from ~0.34 to 0.17 or by 50% (Figure 1A-C). Similar reductions in P_o were observed using lower concentrations of G-1 with 500 $nmol{\cdot}L^{-1}$ and 50 $nmol{\cdot}L^{-1}$ attenuating P_o by ~51% and 35%, respectively (Figure 1D-G). In contrast, the vehicle alone failed to alter BK channel activation (Figure 2). To further characterize the effects of G-1 on BK channel activation, BK mean open and close times were investigated. BK channel mean open times were similar between baseline and G-1 (5 $\mu mol{\cdot}L^{-1}$) conditions (Figure 3A). However, BK mean close times were substantially elevated ~3.6-fold with G-1 administration (Figure 3B). G-1 (5 $\mu mol{\cdot}L^{-1}$) had no effect on single BK channel amplitude in cerebral arterial myocytes (baseline: 10.69 ± 0.19 pA, G-1: 10.73 ± 0.24 pA; n = 8).

To test the effects of G-1 on physiological BK currents, we applied G-1 to isolated arterial myocytes and recorded transient BK currents using whole-cell perforated patch clamp. At a holding potential of −40 mV, transient BK current frequency was similar between groups as G-1 (5 $\mu mol{\cdot}L^{-1}$) elicited both increases (3 cells) and decreases (5 cells) in transient BK current frequency (Figure 4A-B). However, G-1 reduced transient BK current amplitude from ~33.4 to 25.1 pA or by 25% (Figure 4A and C).

To determine the effects of G-1 on arterial myocyte contractility, we performed a G-1 concentration-response curve on pressurized, endothelium-denuded middle cerebral arteries. At 60 $mm{\cdot}Hg^{-1}$ (physiological intravascular pressure), middle cerebral arteries developed 23.5 ± 2.6% (n = 6) myogenic tone and failed to dilate to an acetylholine (ACh, 10 $\mu mol{\cdot}L^{-1}$) challenge consistent with a nonviable endothelium. G-1 elicited negligible vasodilation at nanomolar concentrations with a maximum dilatory effect of 27.3 ± 3.2 μm at 50 $\mu mol{\cdot}L^{-1}$ G-1 (Figure 5A). The application of vehicle alone had no effect on arterial contractility (baseline: 192.8 ± 7.0 μm, vehicle: 194.0 ± 7.1 μm). G-1-mediated vasodilation was gradual with maximum vasodilation established ~4 minutes following G-1 administration. BK channel function was assessed through the administration of iberiotoxin (IBTX, 100 $nmol{\cdot}L^{-1}$), a reversible BK channel pore blocker. IBTX applied alone resulted in a substantial reduction in diameter (vasoconstriction) by ~49.0 μm or by 25% (Figure 5B). In contrast, IBTX added in the presence of the final concentration of G-1 (50 $\mu mol{\cdot}L^{-1}$) elicited a diminished contractile response of ~15.3 μm or by 8%.

4 | DISCUSSION AND CONCLUSIONS

Here, we investigated the effects of G-1, the specific GPER agonist, on BK channel activation in cerebral arterial myocytes. Our

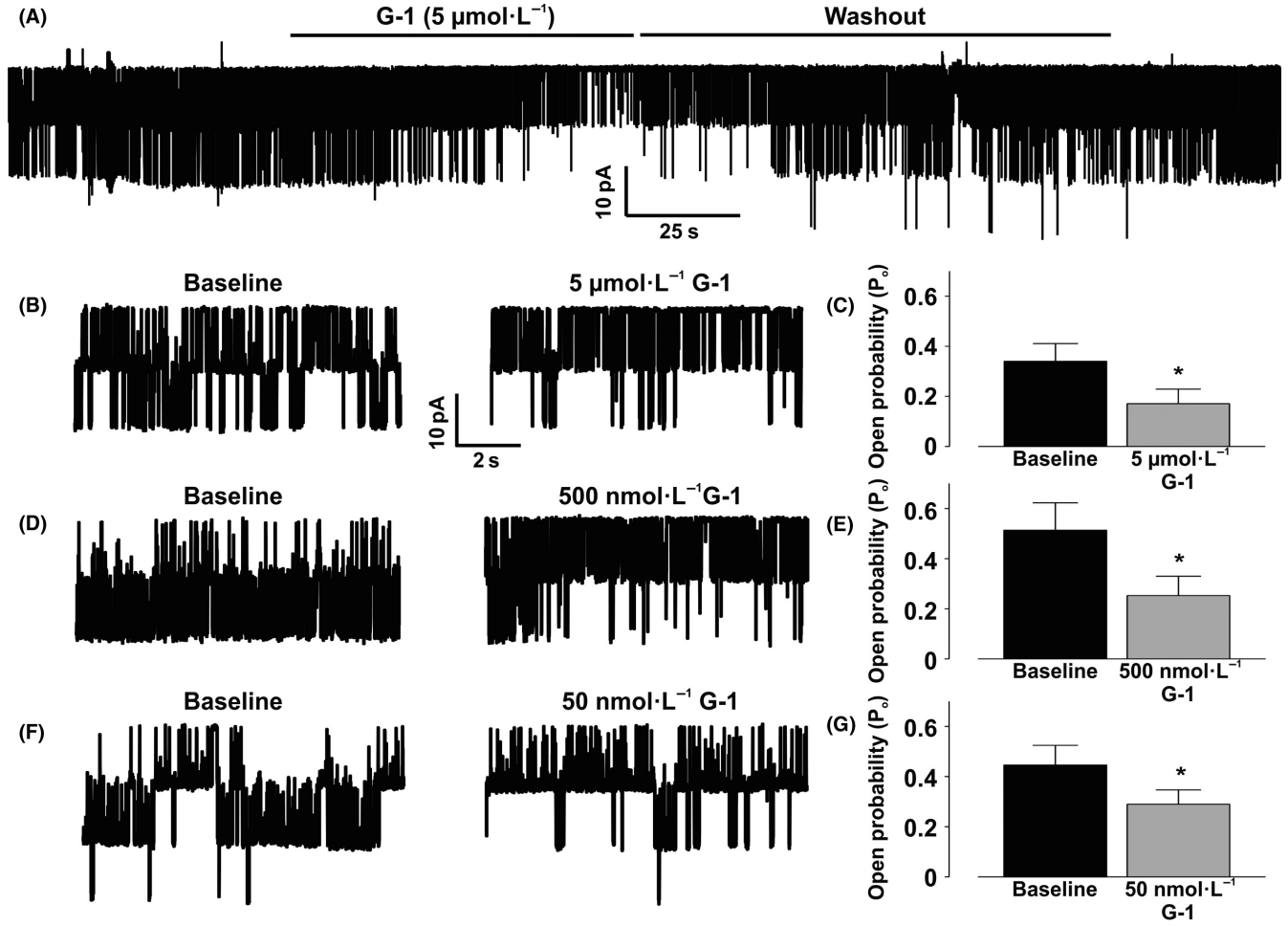

FIGURE 1 G-1 reduces BK channel activation in arterial myocytes. (A) Full-length recording of single BK channel openings demonstrating the effect of G-1 (5 μmol·L^{-1}) on BK channel activation. (B, D, F) Representative BK channel recordings from the same inside-out patches during baseline and G-1 (B: 5 μmol·L^{-1}, D: 500 nmol·L^{-1}, F: 50 nmol·L^{-1}) conditions. (C, E, G) Mean data demonstrating G-1-mediated reduction in BK channel activation at 5 μmol·L^{-1} (C: n = 8 myocytes), 500 nmol·L^{-1} (E: n = 7 myocytes), and 50 nmol·L^{-1} (G: n = 7 myocytes) G-1. *$P < .05$

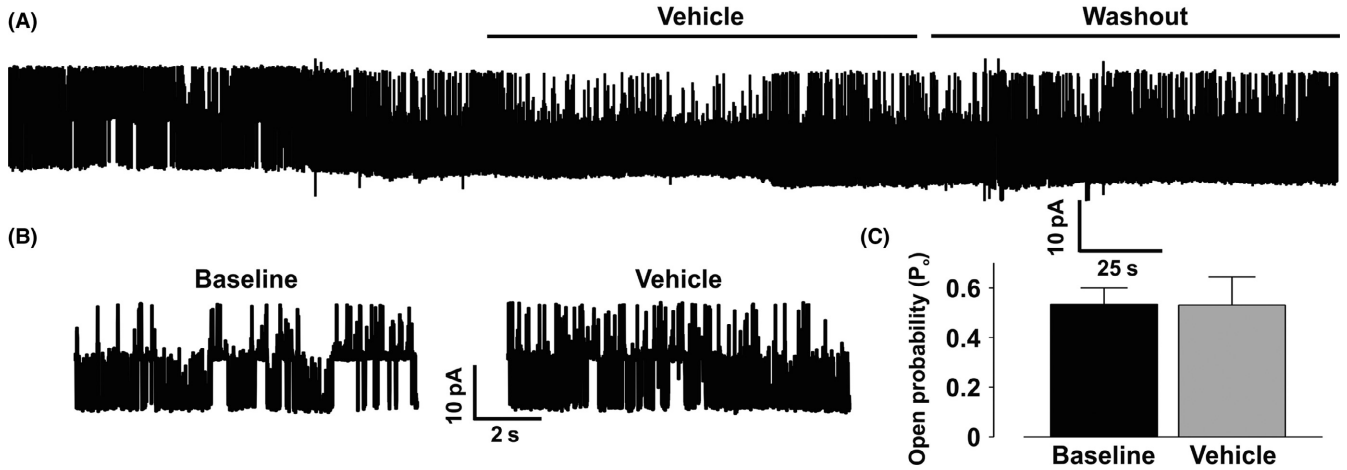

FIGURE 2 Vehicle alone has no effect on BK channel activation in arterial myocytes. (A) Full-length recording of single BK channel openings indicating no effect of vehicle (0.45% EtOH, 0.05% DMSO) on BK channel activation. (B) Representative BK channel recordings from the same inside-out patch during baseline and vehicle conditions. (C) Mean data indicating no change in BK channel activation with addition of the vehicle to the patch bath (n = 6/group)

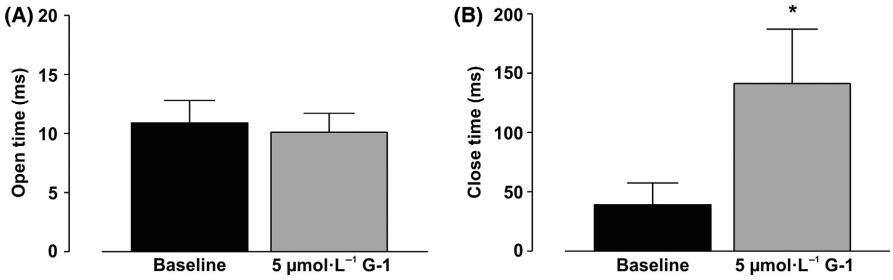

FIGURE 3 G-1 elevates BK channel mean close times in arterial myocytes. (A-B) Mean data indicating BK channel mean open times (A) and mean close times (B) in cerebral arterial myocytes (G-1: 5 μmol·L^{-1}, n = 8/group). *P < .05

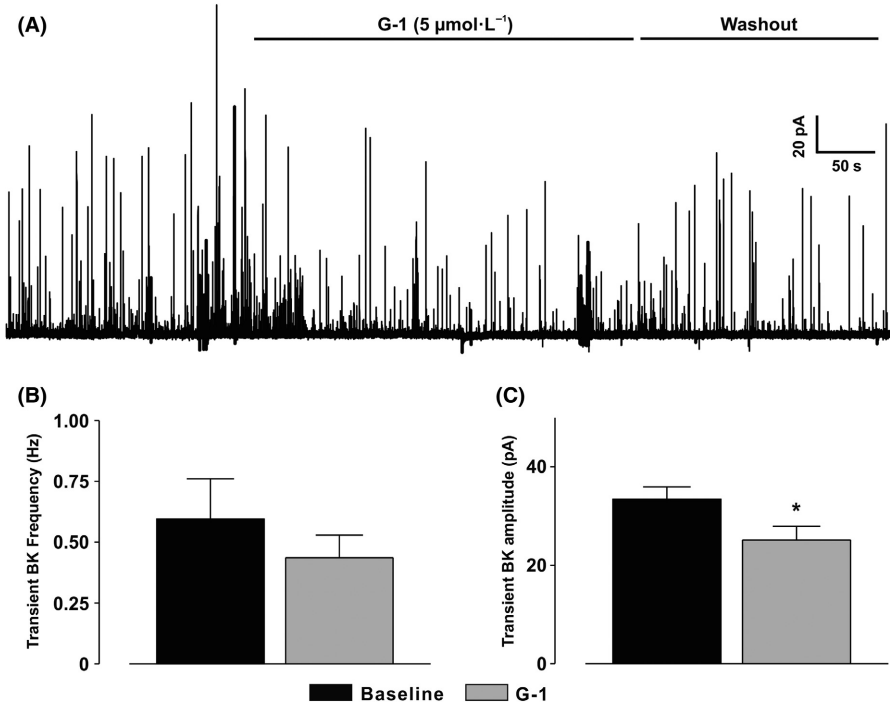

FIGURE 4 G-1 attenuates transient BK currents in arterial myocytes. (A) Representative recording of transient BK currents in cerebral arterial myocytes illustrating the effect of G-1 (5 μmol·L^{-1}) on transient BK currents. (B-C) Mean data demonstrating the effect of G-1 on transient BK current frequency (B) and amplitude (C) in cerebral arterial myocytes (n = 8/group). *P < .05

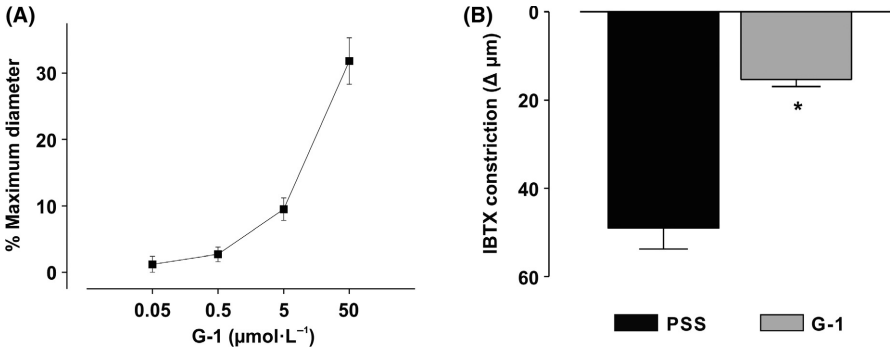

FIGURE 5 G-1 elicits vasodilation, but reduces BK channel function, in cerebral arteries. (A) Cumulative concentration-response curve of G-1 on endothelium-denuded cerebral arteries. (B) Mean data demonstrating the effect of iberiotoxin (IBTX, 100 nmol·L^{-1}), a BK channel pore blocker, on arterial diameter without (PSS) and with G-1 (50 μmol·L^{-1}, n = 6/group). *P < .05

data indicate that G-1 inhibits BK channel activation at nanomolar concentrations through enhancements in BK channel mean close times. G-1 attenuated transient BK current amplitude consistent with its inhibitory effect on single BK channel activation. G-1 elicited vasodilation of pressurized resistance size cerebral arteries at micromolar concentrations, however, BK channel function was reduced in the presence of the GPER agonist. Collectively, these data indicate that G-1-activated GPER elicits endothelium-independent vasodilation in cerebral arteries, but the magnitude is attenuated due to a G-1-mediated reduction in BK channel activation and currents.

G-1 (activator) along with G15 and G36 (inhibitors) are chemicals that were designed to specifically target the GPER receptor without any effects on classical estrogen receptors (e.g., ERα, ERβ).[11,26,27] In binding-affinity studies using recombinant expression of GPER, G-1 exhibited a comparable affinity for GPER as compared to 17β-E2 (G-1: K_i = 11 nmol·L^{-1}, 17β-E2: K_i = 5.7 nmol·L^{-1}).[11] In this salient study, G-1 activated GPER, expressed at the endoplasmic reticulum

of COS7 cells, which initiated a gradual rise of $[Ca^{2+}]_i$ in this cell type. G-1 as well as G15 and G36 have since been utilized to characterize the effects of GPER on physiological and pathophysiological functions of a variety of tissue and cell types.[7,28-31]

GPER is a significant receptor in the maintenance of normal hemodynamics and arterial contractility. Indeed, GPER ablation resulted in an increase in mean arterial pressure further supporting a critical role for this receptor in maintaining normal vascular function.[32] In arterial smooth muscle, G-1 activation of GPER stimulates adenylate cyclase activity, generation of cAMP, and PKA activation.[6,8] Various K^+ channel classes (ATP-sensitive [K_{ATP}], voltage-gated [K_V], and Ca^{2+}-activated [K_{Ca}]) expressed in arterial smooth muscle are activated by the cAMP-PKA pathway,[14] which would suggest a role for these channels with GPER activation. In coronary arteries, the G-1-GPER-mediated cAMP-PKA signaling cascade contributed to a rise in myocyte BK channel function and vasodilation, an effect that was endothelium independent.[9] Moreover, single BK channel activation using inside-out patch clamp did not appear to be affected by G-1 in coronary arterial myocytes. Our data suggest an inhibitory effect of G-1 at nanomolar concentrations on single BK channel activation and BK currents in cerebral arterial myocytes. An explanation for the disparate results and conclusions could lie in the experimental conditions for single-channel analysis. We voltage clamped the membrane potential of excised patches in the physiological range (−40 mV) of cerebral arterial myocytes, whereas the study by Yu et al.[9] voltage clamped their patches at +40 mV.[9] Secondly, we maintained $[Ca^{2+}]_i$ at 10 $\mu mol \cdot L^{-1}$, a concentration in the physiological range of Ca^{2+} sparks,[18] whereas Yu et al. [9] exposed patches to 100 $nmol \cdot L^{-1}$ $[Ca^{2+}]_i$. While basal $[Ca^{2+}]_i$ is maintained at ~100-250 $nmol \cdot L^{-1}$ in pressurized arteries,[33,34] meaningful BK channel activation and BK-mediated vasodilation in pressurized arteries require local $[Ca^{2+}]_i$ concentrations in the micromolar range such as what occurs with Ca^{2+} sparks.[17-19] Thus, using physiological conditions capable of activating BK channels, we were able to detect a substantial reduction in BK channel open probability. In agreement with our single-channel data, G-1 attenuated transient BK current amplitude with variable responses in transient BK current frequency. Transient BK current frequency is coupled to Ca^{2+} spark frequency, the latter influenced by the putative downstream signaling cascade (cAMP-PKA) associated with GPER activation.[8,35] A rise in cAMP-PKA would elicit an increase in Ca^{2+} spark frequency, which would be expected to also increase transient BK current frequency in accordance with the coupling of Ca^{2+} sparks to BK channel activation. Our data suggest that the inhibitory effect of G-1 on BK channel activation may precede measureable outcomes associated with the full signaling cascade associated with GPER, which leads to a relative immediate reduction in transient BK current amplitude with smaller currents no longer meeting the threshold value to be defined as a transient BK current (~10 pA). It is possible that prolonged recordings with G-1 (> 10 minutes) would reveal a uniform increase in transient BK current frequency as the signaling cascade associated with GPER progressed. We did not assess Ca^{2+} spark frequency or amplitude following G-1 administration. It is possible that a portion of the G-1 effect on transient BK currents involves direct effects on RyR channels or other receptors (GPER) at the sarcoplasmic reticulum, which could be an area for future studies. However, our data implicate direct inhibitory effects of G-1 on BK channel activation as a significant mechanism involved in the G-1-mediated reduction in physiological BK currents.

The effects of G-1-GPER on arterial contractility have received a substantial amount of study across multiple vascular beds [5,7] through both wire [8,9,13,36,37] and pressurized [6,38-40] artery myography preparations. Our findings of a vasodilatory role for G-1 in pressurized cerebral resistance size arteries are in agreement with previous studies using myography methods. However, our data suggest that G-1 reduces BK channel function in pressurized cerebral arteries, which would presumably attenuate the magnitude of GPER-mediated vasodilation. While the signaling cascade (cAMP-PKA) with GPER activation is associated with K^+ channel activation,[14] the role of K^+ channels in G-1-GPER-mediated vasodilation remains unclear. In uterine and cerebral resistance size arteries, specific (BK) and non-specific K^+ channel inhibition failed to attenuate the vasodilatory effects of G-1.[39,40] However, BK channel inhibition reduced G-1-mediated relaxation in coronary arteries.[9] In this study, we examined the effects of iberiotoxin, a reversible BK channel pore blocker, on arterial contractility in cerebral arteries, which generate spontaneous myogenic tone in response to an increase in intravascular pressure. Of importance, we investigated the effects of iberiotoxin in the absence and in the presence of G-1 in the same artery to better assess BK channel function under both conditions. BK channel function is an integral component in modulating myogenic tone as evidenced by the robust contractile response following the administration of iberiotoxin with standard PSS. However, the contractile response to iberiotoxin was substantially reduced when administered with G-1. Thus, the G-1-mediated reduction in BK channel activation as observed in patch-clamp experiments mitigates a portion of G-1-mediated vasodilation in pressurized cerebral arteries. Other K^+ channels (K_{ATP}, K_V) sensitive to the GPER signaling cascade (cAMP-PKA) could be responsible for the remaining vasodilation with G-1-mediated activation of GPER in these arteries.

GPER function underlies many of the rapid, nongenomic effects of estrogen.[1,41] Moreover, estrogen receptor antagonists (e.g., fulvestrant) and selective estrogen receptor modulators (tamoxifen, raloxifene) are also capable of GPER activation.[35] Many of the aforementioned estrogens and estrogen-like drugs (fulvestrant, tamoxifen) can also activate BK channels, leading to increased BK channel openings and currents.[20-22] It is particularly interesting that G-1, the only available specific GPER agonist, actually reduces BK channel activation, which in arteries would oppose GPER-mediated vasodilation. These data underscore the difficulty in targeting specific estrogen receptors without initiating off-target effects that could interfere with the desired response. It is also possible that the specific GPER inhibitors, G15 and G36, may also possess some degree of affinity for BK channel subunits as they share structural similarities to G-1.[26,27] Future studies of GPER on vascular function would benefit from the development of new pharmacological tools to

specifically target GPER without the off-target effects that involve proteins (e.g., BK channels) critical to the maintenance of myogenic tone.

In conclusion, G-1, the specific activator for GPER, directly inhibits BK channel activation in cerebral arterial myocytes. G-1 altered BK channel activation through elevated close times, which attenuated physiological BK currents in cerebral arterial myocytes. G-1 elicited vasodilation of endothelium-denuded pressurized cerebral arteries, which occurred despite a reduction in BK channel function. Our data would suggest that GPER is potentially capable of eliciting a greater magnitude of vasodilation, and G-1 may potentially mask the full effect of GPER on arterial contractility.

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AUTHOR CONTRIBUTIONS

K.W.E. developed the experimental design, performed experiments, conducted data analyses, and organized the manuscript. P.G., J.A.G, and M.D.D. all performed experiments and assisted with the manuscript.

DISCLOSURES

The authors declare no conflict of interest with any of the work presented in this manuscript.

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