Published in final edited form as:

Microvasc Res. 2007 July ; 74(1): 39–44.

Myoendothelial Gap Junction Frequency does not Account for Sex Differences in EDHF Responses in Rat MCA

Elke M. Sokoya, Ph.D.^{1,2}, Alan R. Burns, Ph.D.^{2,3}, Sean P. Marrelli, Ph.D.^{1,2,4}, and Jie Chen, M.D., Ph.D.¹

¹ Department of Anesthesiology, Baylor College of Medicine, Houston, Texas, 77030, USA

² Department of Cardiovascular Sciences, Baylor College of Medicine, Houston, Texas, 77030, USA

³ Department of Medicine, Baylor College of Medicine, Houston, Texas, 77030, USA

⁴ Department of Physiology, Baylor College of Medicine, Houston, Texas, 77030, USA

Abstract

Previous findings from our laboratory have shown that dilations to endothelium-derived hyperpolarizing factor (EDHF) in rat middle cerebral artery (MCA) are less in females compared to males. Myoendothelial gap junctions (MEGJs) appear to mediate the transfer of hyperpolarization between endothelium and smooth muscle in males. In the present study we hypothesized that MEGJs are the site along the EDHF pathway which is compromised in female rat MCA.

Membrane potential in endothelium was measured using the voltage-sensitive dye di-8-ANEPPS and in smooth muscle using intracellular glass microelectrodes in the presence of L-NAME (3×10^{-5} M) and indomethacin (10^{-5} M). Electron microscopy was used to assess MEGJ characteristics. In endothelial cells, the di-8-ANEPPS fluorescence ratio change to 10^{-5} M UTP was similar in males ($-2.9 \pm 0.5\%$) and females ($-3.2 \pm 0.2\%$), indicating comparable degrees of endothelial cell hyperpolarization. However, smooth muscle cell hyperpolarization to 10^{-5} M UTP was significantly attenuated in females (0mV hyperpolarization; -31 ± 1.5 mV resting) compared to males (8mV hyperpolarization; -28 ± 1.7 mV resting). Ultrastructural evidence suggested that MEGJ frequency and area of contact were comparable between males and females. Taken together, our data suggest that in rat MCA, MEGJ frequency does not account for the reduced EDHF responses observed in females compared to males. We conclude that reduced myoendothelial coupling and/or homocellular coupling within the media may account for these differences.

Keywords

endothelium-derived hyperpolarizing factor; gap junctions; sex; smooth muscle

Introduction

The mechanism defining endothelium-derived hyperpolarizing factor (EDHF)-mediated dilations in the vasculature still remains largely unresolved. As a complicating factor, the mechanism appears to be specific to the vascular bed (Dong et al., 2000), age (Fujii et al.,

Corresponding author: Elke M. Sokoya, Ph.D., Department of Anesthesiology, One Baylor Plaza, Suite 433D, Houston, Texas, 77030, USA, Phone: (713) 798-7724, Fax: (713) 798-7644, Email: egolding@bcm.tmc.edu

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1993), species (Triggle et al., 1999), and pathological condition (Fukao et al., 1997;Golding et al., 2001). Nonetheless, there are certain hallmark characteristics of EDHF that stand alone (Busse et al., 2002;Feletou and Vanhoutte, 2006). Namely, it is independent from both nitric oxide (NO) and prostacyclin, it requires activation of endothelial cell (EC) calcium-sensitive potassium (K_{Ca}) channels, and it culminates in smooth muscle cell (SMC) hyperpolarization (Golding et al., 2002). Many 'factors' have been ascribed to the actions of EDHF including K^+ (Edwards et al., 1998), 11,12 epoxyeicosatrienoic acid (Campbell et al., 1996;Fisslthaler et al., 1999), hydrogen peroxide (Matoba et al., 2000) and C-type natriuretic peptide (Chauhan et al., 2003). However in some arteries, EDHF does not appear to be solely attributed to a diffusible endothelial factor, leading to speculation that direct electrical communication between the endothelium and smooth muscle may exist (Sandow and Hill, 2000). Indeed, recent studies in rat middle cerebral artery (MCA) have implicated a role for intercellular channels called gap junctions in mediating EDHF responses (Sokoya et al., 2006;McNeish et al., 2006). In this way, EC hyperpolarization may be communicated directly to the underlying SMC via myoendothelial gap junctions (MEGJs) (Sandow et al., 2002).

To add to the heterogeneity of the EDHF response, we have shown that sex is a contributing factor. In the rat MCA, vasodilations to EDHF are less in females than in males (Golding and Kepler, 2001). The reasoning behind this is largely unknown although previous studies suggest that intermediate KCa channels are not involved (Sokoya et al., 2007) and the site has been localized to being downstream from EC calcium and upstream from SMC hyperpolarization (Golding et al., 2002).

In the present study, we hypothesized that MEGJs are the site along the EDHF pathway which is compromised in female rat MCA. To address this hypothesis we assessed both function and structure of MEGJs in male and female rat MCAs by measuring EC and SMC hyperpolarization during EDHF stimulation and examining MEGJ frequency, respectively. Our findings revealed that while EC hyperpolarization was comparable, SMC hyperpolarization was attenuated in females compared to males. MEGJ area and frequency was comparable between males and females. We conclude that reduced myoendothelial coupling and/or SMC homocellular coupling may account for the attenuated EDHF-mediated SMC hyperpolarization in females.

Materials and Methods

Experiments were carried out in accordance with the NIH guidelines for the care and use of laboratory animals and were approved by the Animal Protocol Review Committee at Baylor College of Medicine. Rats were housed under a 12 h light/12 h dark cycle with unrestricted access to food and water. Experiments were performed on age-matched (70–90 days old) male (n=17) and female (n=17) Long-Evans rats.

Harvesting and Mounting Cerebral Vessels

Animals were allowed to spontaneously breathe isoflurane in an anesthetic chamber and were then decapitated. The brain was removed and placed in cold physiological salt solution (PSS). The MCA was harvested, cleaned of surrounding connective tissue and cannulated with micropipettes in a vessel chamber. PSS was circulated abluminally through a heat-exchanger in order to maintain the bath temperature at 37°C. Intraluminal pressure was monitored via in-line transducers, which were connected to two strain gauge panel meters (Omega, Stamford, CT). Once mounted, vessels were tested for leaks and those that did not maintain a steady pressure were discarded. The vessel chamber was mounted on the stage of an inverted microscope. Transmural pressure was set at 85 mmHg with a flow of 150 ul/min through the lumen, and the vessels allowed to equilibrate for 1 h. During this time the vessels constricted from their fully dilated diameter at initial pressurization. Vessels that did not develop this 'spontaneous tone' were deemed non-viable and discarded.

Measurement of Endothelial Cell Membrane Potential Changes

Following the development of spontaneous tone in pressurized MCAs, the luminal and abluminal compartments were exposed to L-NAME ($3 \times 10^{-5}\text{M}$) and indomethacin (10^{-5}M) for 30 mins to inhibit nitric oxide synthase and cyclooxygenase, respectively. EC membrane potential changes were measured using a voltage-sensitive dye, 4-{2-[6-(dioctylamino)-2-naphthalenyl]ethenyl}1-(3-sulfopropyl)-pyridinium (di-8-ANEPPS) (Beach et al., 1996; Marrelli et al., 2003). The ratio of the 560 and 620 emissions changes with endothelial membrane potential thereby offering a qualitative measurement of EC membrane potential changes. In order to reduce the potential artifact of changes in fluorescence intensity due to vasodilation, MCAs were pre-dilated using verapamil (3uM, abluminally). Dye was selectively loaded into the endothelium by perfusing the lumen of the MCAs with di-8-ANEPPS (10 uM/0.1% pluronic) for 20 min followed by a 20 min washout period. Using this method, previous studies using confocal microscopy have shown that the dye is confined to the endothelium (Marrelli et al., 2003). UTP (10^{-5}M) was specifically applied to the lumen for 5 min and then washed out with PSS. Prior work has shown that this concentration of UTP produces a maximum dilation of male rat MCA (You et al., 1997).

Measurement of Smooth Muscle Cell Membrane Potential

The MCA was isolated, cut open longitudinally and pinned to the base of a vessel chamber with the smooth muscle facing upwards. The preparation was superfused with PSS. The vessels were exposed to L-NAME ($3 \times 10^{-5}\text{M}$) and indomethacin (10^{-5}M) for 30 mins. Membrane potential was recorded according to the protocol previously described (Sandow et al., 2002). Briefly, intracellular glass microelectrodes with resistances of 100 to 150 M Ω were filled with 2% Lucifer Yellow and 1M KCl. During impalement, the Lucifer Yellow diffused into the cell, thereby confirming identification of the impaled cell. Membrane potential changes to UTP (10^{-5}M) or KCl (15mM) were then recorded in the smooth muscle.

Electron Microscopy

MCAs were processed for electron microscopy as previously described (Sokoya et al., 2006). Briefly, anesthetized male and female rats were perfusion fixed with Sorenson's PBS containing 3% glutaraldehyde. The brain was removed and immersed in this fixative overnight at 4°C. The following day, the MCA was dissected from the brain and placed in PBS at 4°C. Tissue samples were post-fixed in 1% tannic acid (5 min) followed by 1% osmium tetroxide (1 h) and then aqueous uranyl acetate (1 h). Samples were subsequently dehydrated in a graded ethanol series, embedded in Araldite resin and ultrathin serial sections (~100 nm) were obtained using an ultramicrotome (RMC 7000, RMC, AZ) equipped with a diamond knife. Sections were stained with uranyl acetate and lead citrate before viewing with a JEOL 200CX electron microscope.

Myoendothelial gap junctions (MEGJs) were identified as a breach in the basal laminae of both the EC and SMC through which cell protrusions extended such that the distance between the EC and SMC plasma membranes was $\leq 3.5\text{nm}$. MEGJs displayed the characteristic pentalaminar membrane structure where the central region had a higher electron opacity than the inner leaflets. MEGJ area was determined as the length of membrane contact multiplied by the thickness (100 nm) of the section multiplied by the number of sections in which it appeared. The maximum diameter of the MCAs was calculated from circumference measurements (using the public domain NIH Image J Program) made on thick (0.5um) sections stained with toluidine blue and viewed on a light microscope.

En Face Whole Mounts

Male and female rats were perfused via the left ventricle with 0.2% silver nitrate solution. The MCA was then dissected from the brain, cut open longitudinally with the endothelium facing upwards, and placed on a microscope slide which was then coverslipped. EC borders, revealed by silver nitrate deposition, allowed measurements of EC area, perimeter, length, and maximum width in *en face* whole mounts using MetaVue software (Universal Imaging Corporation, PA).

Drugs and Solutions

Stock solutions of UTP (10^{-2} M) and L-NAME (3×10^{-2} M) were prepared in distilled water, aliquotted and then frozen. A stock solution of indomethacin (10^{-2} M) was prepared in a solution of Na_2CO_3 and distilled water (1:1 by weight).

Data Analysis and Calculations

All data are presented as means $\pm$ SEM. For the EC and SMC membrane potential data, statistical significance was tested using a repeated measures ANOVA and multiple comparisons were made using a Student-Newman-Keuls test. For the MEGJ data, statistical significance was tested using a t-test. Differences were considered significant at error probabilities less than 0.05 ($P < 0.05$).

Results

Endothelial Cell Membrane Potential

In the presence of L-NAME and indomethacin, the di-8-ANEPPS fluorescence ratio change to 10^{-5} M UTP was similar in males ($-2.9 \pm 0.5\%$; $n=4$) and females ($-3.2 \pm 0.2\%$; $n=4$), indicating comparable degrees of endothelial cell hyperpolarization (Figure 1). Although there was a tendency for females to be more hyperpolarized in the presence of UTP, this did not reach statistical significance ($p=\text{ns}$; 2-way RM ANOVA).

Smooth Muscle Cell Membrane Potential

Resting membrane potential of smooth muscle in rat MCA was comparable in males ($-39 \pm 1.2\text{mV}$; $n=7$) and females ($-41 \pm 0.9\text{mV}$; $n=7$). Exposure to L-NAME (3×10^{-5} M) and indomethacin (10^{-5} M) depolarized SMC to similar levels in males ($-28 \pm 1.7\text{mV}$; $n=7$) and females ($-31 \pm 1.5\text{mV}$; $n=7$).

Stimulation of the endothelium with 10^{-5} M UTP hyperpolarized SMC of males ($-28 \pm 1.7\text{mV}$ pre-UTP and $-36 \pm 1.8\text{mV}$ post-UTP; $n=7$), but failed to evoke a hyperpolarization in SMC of females ($-31 \pm 1.5\text{mV}$ pre-UTP and $-31 \pm 1.6\text{mV}$ post-UTP; $n=7$; Figure 2). We have previously shown that SMC hyperpolarization to UTP in males is abolished in the presence of charybdotoxin and apamin, in support of the role of UTP in stimulating an EDHF response (Sokoya et al., 2006). In response to 15mM KCl however, a hyperpolarization was observed in both male ($-12 \pm 1\text{mV}$; $n=7$) and female ($-10 \pm 1\text{mV}$; $n=7$) SMCs (Figure 2).

MCA and MEGJ Morphology

In toluidine blue-stained cross-sections, MCA diameter was comparable between males ($192.2 \pm 0.7\mu\text{m}$; $n=19$) and females ($192.9 \pm 2.6\mu\text{m}$; $n=25$). Furthermore, in silver nitrate stained *en face* whole mounts, EC dimensions were comparable between males and females (Table 1).

MEGJs were observed in both male and female rat MCA (Figure 3). The frequency of MEGJs in rat MCA examined by electron microscopy was comparable between males and females. In male rat MCA ($n=3$), MEGJs occurred with a frequency of 5 MEGJ in 116 cross-sections (2.0

± 0.5 MEGJ/5 μ m vessel length). Analysis of female rat MCA (n=3) revealed 5 MEGJ in 111 cross-sections (2.1 ± 1.4 MEGJ/5 μ m vessel length). The mean total area of contact between smooth muscle and endothelium at these MEGJ contact regions was also similar between males ($0.09 \pm 0.03\mu\text{m}^2$) and females ($0.08 \pm 0.05\mu\text{m}^2$).

Discussion

The purpose of the present study was to further localize the site along the EDHF pathway which is compromised in female rat MCA. Herein we present three new findings. First, EC hyperpolarization to UTP was similar in both males and females. Second, SMC hyperpolarization to UTP was significantly attenuated in females compared to males. Third, MEGJ frequency and area of contact was comparable in males and females.

In order to assess changes in EC hyperpolarization, we used the membrane potential-sensitive dye, di-8-ANEPPS. Although the use of such a ratiometric dye does not provide us with quantitative measurements, it does indicate that the amount of EC hyperpolarization is comparable in males and females. Our data show a 3% decrease in the fluorescence ratio in response to 10^{-5} M UTP which would be equivalent to a hyperpolarization of approximately 20–30mV (Beach et al., 1996). This is consistent with the membrane potential changes obtained using sharp electrodes in the pinned out MCA (Sokoya et al., 2006).

Sharp electrode recordings from rat MCA demonstrated that SMC hyperpolarization in response to UTP was significantly reduced in females compared to males (Figure 2). However hyperpolarization in response to 15mM KCl was comparable in males and females. These data are in line with our previous findings that 15mM KCl elicited a comparable vasodilation in males and females (Golding et al., 2002). At this concentration, KCl directly stimulates inward-rectifying potassium channels located on the smooth muscle of the rat MCA, with no contributing role of the endothelium (Johnson et al., 1998). Therefore, the resulting SMC hyperpolarization from UTP and KCl differs in that the former relies on EC hyperpolarization while the latter does not. Thus, our results suggest that MCA from females can respond appropriately to hyperpolarization of the smooth muscle.

We have previously shown that dilations to EDHF in males are significantly attenuated in the presence of gap mimetic peptides (small peptides that putatively interfere with the extracellular domain of connexins) (Sokoya et al., 2006) and ovariectomized females (unpublished observations). In the present study we have shown that during EDHF stimulation, EC hyperpolarization was sustained, while SMC hyperpolarization was attenuated in female compared to male rat MCA. Two possibilities could explain these findings. First, at the MEGJ level, females may have a reduced number of gap junctions between endothelium and smooth muscle (MEGJs). However our morphological evidence does not support this possibility since MEGJ frequency was comparable between males and females. Furthermore, MCA diameter and EC dimensions were similar between the two groups. While the number of MEGJs is not a contributing factor, it is possible that the conductance of MEGJs during EDHF stimulation may be compromised in females. The electrical conductance of the junctional membrane depends upon many factors including the number of junctional channels, the conductance of a single channel, and the open probability of individual channels. Interestingly sex differences have been shown with respect to neuronal cell coupling (Hatton and Yang, 1990) and further studies have shown that neuronal dye coupling is low when circulating plasma estradiol titer is high (Hatton and Zhao Yang, 2002). While we attempted to directly assess myoendothelial gap junction conductance using the dye calcein-AM, these studies were unsuccessful. Future work will need to address the possibility that sex may be a contributing factor in modulating the electrical conductance of MEGJ channels.

The second possibility is that frequency and/or conductance of homocellular gap junctions between smooth muscle cells is reduced in females. Due to the complexity and vast time commitment associated with navigating three layers of smooth muscle cells in serial cross-sections, we did not complete a quantitative analysis of gap junctions within the media of rat MCA. Notably, no quantitation of such gap junctions exists in the literature. Regardless of whether the frequency of SMC-SMC gap junctions differs between the male and female, there remains the possibility that conductance is compromised. In response to the endothelium dependent agonist UTP, hyperpolarization within the SMCs was assessed in the outermost layer, closest to the adventitia. This raises the possibility that the reduced hyperpolarization observed in the SMCs, occurs at the level of homocellular coupling within the media. There is also the possibility that the connexin protein expression profile at the heterocellular and/or homocellular gap junction level is different in females compared to males. This may underlie the potential differences in gap junction conductance as outlined above.

To date, no consensus has been reached as to whether a diffusible factor is involved in EDHF responses in the rat MCA. Some groups have reported attenuated EDHF responses in the presence of barium and/or ouabain (to abrogate any hyperpolarization due to the activation of inward rectifier potassium channels and Na^+/K^+ ATPase, respectively) (McNeish et al., 2005) while others have not (Johnson et al., 1998; You et al., 1999). Regardless of whether K^+ is an important aspect of EDHF responses, our data do not support a role for K^+ in attributing to the reduced EDHF responses in females (see above). Activation of EC phospholipase A_2 has been shown to be a key step in mediating the EDHF response (You et al., 2002) although further studies have ruled out an involvement of arachidonic acid metabolites (You et al., 2005). Interestingly, arachidonic acid (the main product of reactions catalyzed by phospholipase A_2) has been shown to block dye coupling between retinal horizontal cells (Miyachi et al., 1994).

In summary, we have shown that during EDHF responses in rat MCA, hyperpolarization to UTP is significantly attenuated in SMC but not EC of females compared to males. This did not appear to be accounted for by MEGJ morphology, since both frequency and contact area were comparable in males and females. Taken together, our data suggest that other factors such as reduced myoendothelial coupling and/or homocellular coupling within the media account for the reduced EDHF response in females compared to males.

Acknowledgements

This work was supported by the National Institutes of Health HL72954 (EMS), and HL-070537 (ARB) and the American Heart Association 0230353N (SPM) and 0665100Y (SPM). We are grateful to Evelyn Brown and Sharon Phillips for their excellent technical assistance.

References

1. Beach JM, McGahren ED, Xia J, Duling BR. Ratiometric measurement of endothelial depolarization in arterioles with a potential-sensitive dye. *Am J Physiol* 1996;270:H2216–2227. [PubMed: 8764277]
2. Busse R, Edwards G, Feletou M, Fleming I, Vanhoutte PM, Weston AH. EDHF: bringing the concepts together. *Trends Pharmacol Sci* 2002;23:374–380. [PubMed: 12377579]
3. Campbell WB, Gebremedhin D, Pratt PF, Harder DR. Identification of epoxyeicosatrienoic acids as endothelium-derived hyperpolarizing factors. *Circ Res* 1996;78:415–423. [PubMed: 8593700]
4. Chauhan SD, Nilsson H, Ahluwalia A, Hobbs AJ. Release of C-type natriuretic peptide accounts for the biological activity of endothelium-derived hyperpolarizing factor. *Proc Natl Acad Sci U S A*. 2003
5. Dong H, Jiang Y, Cole WC, Trigg CR. Comparison of the pharmacological properties of EDHF-mediated vasorelaxation in guinea-pig cerebral and mesenteric resistance vessels. *Br J Pharmacol* 2000;130:1983–1991. [PubMed: 10952691]
6. Edwards G, Dora KA, Gardener MJ, Garland CJ, Weston AH. K^+ is an endothelium-derived hyperpolarizing factor in rat arteries. *Nature* 1998;396:269–272. [PubMed: 9834033]

7. Feletou M, Vanhoutte PM. Endothelium-derived hyperpolarizing factor: where are we now? *Arterioscler Thromb Vasc Biol* 2006;26:1215–1225. [PubMed: 16543495]
8. Fisslthaler B, Popp R, Kiss L, Potente M, Harder DR, Fleming I, Busse R. Cytochrome P450 2C is an EDHF synthase in coronary arteries. *Nature* 1999;401:493–497. [PubMed: 10519554]
9. Fujii K, Ohmori S, Tominaga M, Abe I, Takata Y, Ohya Y, Kobayashi K, Fujishima M. Age-related changes in endothelium-dependent hyperpolarization in the rat mesenteric artery. *Am J Physiol* 1993;265:H509–516. [PubMed: 8368354]
10. Fukao M, Hattori Y, Kanno M, Sakuma I, Kitabatake A. Alterations in endothelium-dependent hyperpolarization and relaxation in mesenteric arteries from streptozotocin-induced diabetic rats. *Br J Pharmacol* 1997;121:1383–1391. [PubMed: 9257918]
11. Golding EM, Ferens DM, Marrelli SP. Altered calcium dynamics do not account for attenuation of endothelium-derived hyperpolarizing factor-mediated dilations in the female middle cerebral artery. *Stroke* 2002;33:2972–2977. [PubMed: 12468799]
12. Golding EM, Kepler TE. Role of estrogen in modulating EDHF-mediated dilations in the female rat middle cerebral artery. *Am J Physiol Heart Circ Physiol* 2001;280:H2417–2423. [PubMed: 11356593]
13. Golding EM, Marrelli SP, You J, Bryan RM Jr. Endothelium-derived hyperpolarizing factor in the brain: a new regulator of cerebral blood flow? *Stroke* 2002;33:661–663. [PubMed: 11872883]
14. Golding EM, You J, Robertson CS, Bryan RM Jr. Potentiated endothelium-derived hyperpolarizing factor-mediated dilations in cerebral arteries following mild head injury. *J Neurotrauma* 2001;18:691–697. [PubMed: 11497095]
15. Hatton GI, Yang QZ. Activation of excitatory amino acid inputs to supraoptic neurons. I. Induced increases in dye-coupling in lactating, but not virgin or male rats. *Brain Res* 1990;513:264–269. [PubMed: 2161699]
16. Hatton GI, Zhao Yang Q. Peripartum interneuronal coupling in the supraoptic nucleus. *Brain Research* 2002;932:120–123. [PubMed: 11911868]
17. Johnson TD, Marrelli SP, Steenberg ML, Childres WF, Bryan RM Jr. Inward rectifier potassium channels in the rat middle cerebral artery. *Am J Physiol* 1998;274:R541–R547. [PubMed: 9486315]
18. Marrelli SP, Eckmann MS, Hunte MS. Role of endothelial intermediate conductance K_{Ca} channels in cerebral EDHF-mediated dilations. *Am J Physiol Heart Circ Physiol* 2003;285:H1590–1599. [PubMed: 12805022]
19. Matoba T, Shimokawa H, Nakashima M, Hirakawa Y, Mukai Y, Hirano K, Kanaide H, Takeshita A. Hydrogen peroxide is an endothelium-derived hyperpolarizing factor in mice. *J Clin Invest* 2000;106:1521–1530. [PubMed: 11120759]
20. McNeish AJ, Dora KA, Garland CJ. Possible role for K⁺ in endothelium-derived hyperpolarizing factor-linked dilatation in rat middle cerebral artery. *Stroke* 2005;36:1526–1532. [PubMed: 15933259]
21. McNeish AJ, Sandow SL, Neylon CB, Chen MX, Dora KA, Garland CJ. Evidence for involvement of both IK_{Ca} and SK_{Ca} channels in hyperpolarizing responses of the rat middle cerebral artery. *Stroke* 2006;37:1277–1282. [PubMed: 16556879]
22. Miyachi E, Kato C, Nakaki T. Arachidonic acid blocks gap junctions between retinal horizontal cells. *Neuroreport* 1994;5:485–488. [PubMed: 7911687]
23. Sandow SL, Hill CE. Incidence of myoendothelial gap junctions in the proximal and distal mesenteric arteries of the rat is suggestive of a role in endothelium-derived hyperpolarizing factor-mediated responses. *Circ Res* 2000;86:341–346. [PubMed: 10679487]
24. Sandow SL, Tare M, Coleman HA, Hill CE, Parkington HC. Involvement of myoendothelial gap junctions in the actions of endothelium-derived hyperpolarizing factor. *Circ Res* 2002;90:1108–1113. [PubMed: 12039801]
25. Sokoya EM, Burns AR, Setiawan CT, Coleman HA, Parkington HC, Tare M. Evidence for the involvement of myoendothelial gap junctions in EDHF-mediated relaxation in the rat middle cerebral artery. *Am J Physiol Heart Circ Physiol* 2006;291:H385–393. [PubMed: 16443675]
26. Sokoya EM, You J, Chen J. DCEBIO-Mediated Dilations Are Attenuated in the Female Rat Middle Cerebral Artery. *J Vasc Res* 2007;44:169–174. [PubMed: 17308435]

27. Triggle CR, Dong H, Waldron GJ, Cole WC. Endothelium-derived hyperpolarizing factor(s): species and tissue heterogeneity. *Clin Exp Pharmacol Physiol* 1999;26:176–179. [PubMed: 10065343]
28. You J, Golding EM, Bryan RM Jr. Arachidonic acid metabolites, hydrogen peroxide, and EDHF in cerebral arteries. *Am J Physiol Heart Circ Physiol* 2005;289:H1077–1083. [PubMed: 15863454]
29. You J, Johnson TD, Childres WF, Bryan RM Jr. Endothelial-mediated dilations of rat middle cerebral arteries by ATP and ADP. *Am J Physiol* 1997;273:H1472–H1477. [PubMed: 9321839]
30. You J, Johnson TD, Marrelli SP, Mombouli JV, Bryan RM Jr. P2u receptor-mediated release of endothelium-derived relaxing factor/nitric oxide and endothelium-derived hyperpolarizing factor from cerebrovascular endothelium in rats. *Stroke* 1999;30:1125–1133. [PubMed: 10229754]
31. You J, Marrelli SP, Bryan RM Jr. Role of cytoplasmic phospholipase A2 in endothelium-derived hyperpolarizing factor dilations of rat middle cerebral arteries. *J Cereb Blood Flow Metab* 2002;22:1239–1247. [PubMed: 12368663]

Figure 1.

Endothelial cell membrane potential responses to 10^{-5} M UTP in the presence of L-NAME (3×10^{-5} M) and indomethacin (10^{-5} M) in males (open circles; $n=4$) and females (filled circles; $n=4$). Endothelial cells were selectively loaded with the fluorescent dye di-8-ANEPPS (10 μ M). The resulting ratio of the 560 and 620 emissions (F₅₆₀/F₆₂₀) reflects membrane potential changes in the endothelium. Verapamil (3 μ M, abluminally) was used to pre-dilate the artery in order to eliminate any potential artifact of changes in fluorescence intensity due to vasodilation.

Figure 2.

Summary of sharp electrode recordings of smooth muscle cell membrane potential changes in male (filled bars) and female (open bars) rat MCA (A). SMC hyperpolarization to 10^{-5} M UTP was significantly attenuated in females ($n=7$) compared to males ($n=7$; * $P<0.05$). Smooth muscle cell hyperpolarization to 15mM KCl was similar in both males and females.

Representative tracings depicting the SMC membrane potential changes in response to 10^{-5} M UTP in males (B) and females (C). All experiments were performed in the presence of L-NAME (3×10^{-5} M) and indomethacin (10^{-5} M).

Figure 3.

Transmission electron micrograph of a female rat MCA showing a myoendothelial gap junction (MEGJ). An endothelial cell (EC) projection extends through the internal elastic lamina (IEL) towards the smooth muscle cell (SMC) where they meet (arrow). Scale bar, 250nm. The inset shows a higher magnification of the MEGJ where one can discern the pentalaminar membrane structure characteristic of gap junctions. Scale bar, 50nm.

Table 1

Endothelial cell area, perimeter, length and maximum width in male and female rat middle cerebral arteries.

Endothelial cell dimensions were comparable between male and female MCAs.

Endothelial Cell	Males	Females
Area (μm^2)	302 $\pm$ 4	287 $\pm$ 5
Perimeter (μm)	145 $\pm$ 2	151 $\pm$ 2
Length (μm)	72 $\pm$ 1	71 $\pm$ 1
Maximum Width (μm)	8 $\pm$ 0.2	7 $\pm$ 0.2