

Evidence of Reduced Myoendothelial Coupling in Female Rat Middle Cerebral Artery during EDHF Stimulation

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INTRODUCTION

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. Since myoendothelial gap junctions (MEGJs) appear to mediate the transfer of hyperpolarization between endothelium and smooth muscle in males (Sokoya et al, Am J Physiol, 2006), we hypothesized that reduced myoendothelial coupling accounts for the attenuated EDHF response in female rat MCA. To address this hypothesis we assessed endothelial cell (EC) and smooth muscle cell (SMC) hyperpolarization during EDHF stimulation in males and females.

RESULTS

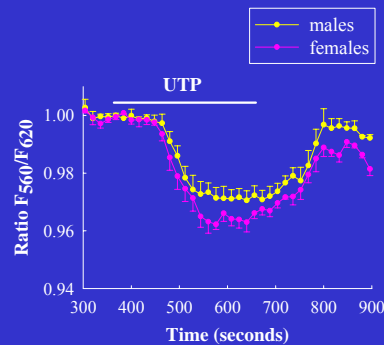


Figure 2. In the presence of L-NAME and indomethacin, the di-8-ANEPPS fluorescence ratio change to 10^{-5} M UTP was similar in males ($-2.9 \pm 0.5\%$; $n=4$) and females ($-3.2 \pm 0.2\%$; $n=4$), indicating comparable degrees of endothelial cell hyperpolarization.

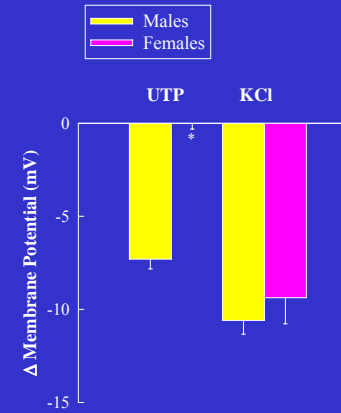


Figure 4. Stimulation of the endothelium with 10^{-5} M UTP hyperpolarized SMC of males, but failed to evoke a hyperpolarization in SMC of females. In response to 15mM KCl, however, a hyperpolarization was observed in both male and female SMCs.

Table 1. Properties of MEGJs and endothelial cells (ECs) in male and female rat MCAs. MEGJ frequency, MEGJ area and endothelial cell dimensions were comparable between male and female MCAs.

	Males	Females
MEGJ/5um vessel length	2.0±0.5	2.1±1.4
MEGJ area (μm^2)	0.09±0.03	0.08±0.05
EC Area (μm^2)	302±4	287±5
EC Perimeter (μm)	145±2	151±2
EC Length (μm)	72±1	71±1
EC Maximum Width (μm)	8±0.2	7±0.2

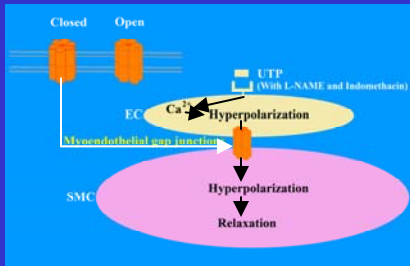


Figure 1. Diagram of the myoendothelial gap junction involvement in EDHF-mediated dilation.

METHODS

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 MEGJs in 100-nm cross-sections. Myoendothelial gap junctions (MEGJs) were identified as a breach in the basal laminae of both the EC and SMC where both cell types were a distance of ≤ 3 nm. MEGJ area was determined as the length of the contact multiplied by the thickness of the tissue sampled. EC area, perimeter, length, and width were measured in *en face* whole mounts stained with silver nitrate.

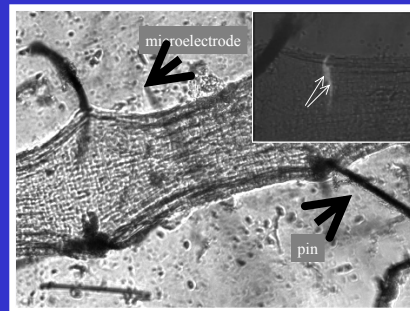


Figure 3. Membrane potential recordings in smooth muscle cells were measured in pinned out MCAs using intracellular glass microelectrodes (60-80 mega ohm resistance). Inset: recordings from a smooth muscle cell were confirmed by Lucifer Yellow injection.

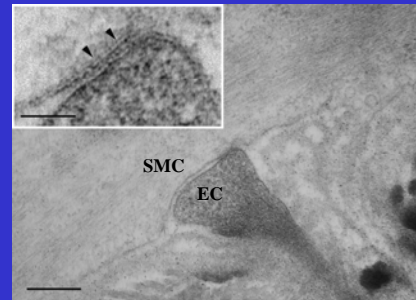


Figure 5. Transmission electron micrograph of a female rat MCA showing a myoendothelial gap junction. An endothelial cell (EC) projection extends through the internal elastic lamina towards the smooth muscle cell (SMC) where they meet (arrow). Scale bar, 250nm. Inset Scale bar, 50nm.

CONCLUSIONS

We have shown that during an EDHF response 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.

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.

References

Sokoya et al, Am J Physiol. 291(1): H385-93, 2006.

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