Sex-Specific Endothelial Dysfunction after Focal Cerebral Ischemia

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Inroduction

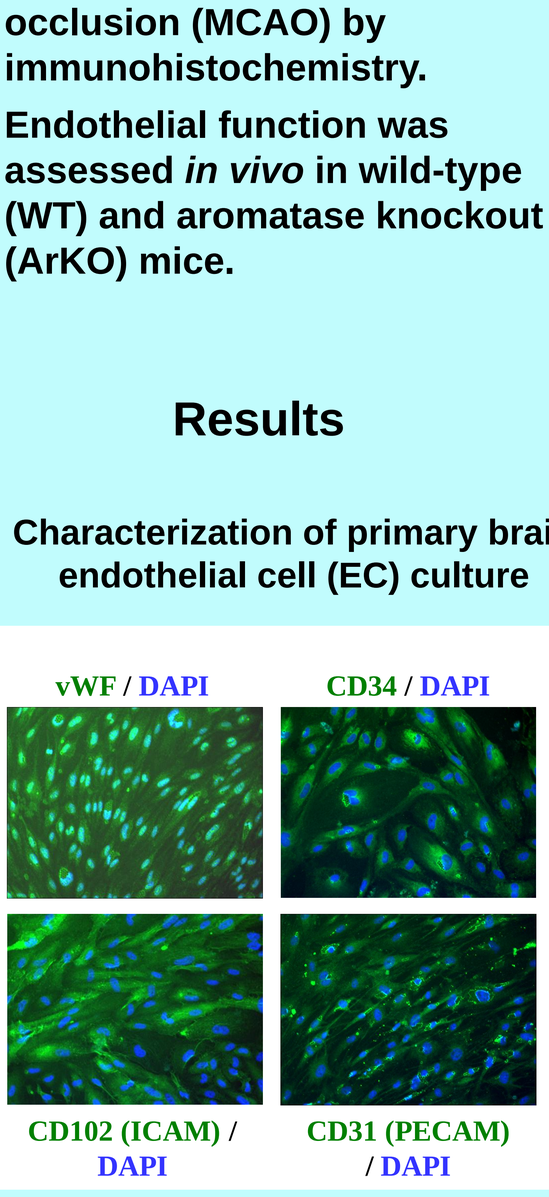
- Sex differences in endothelial function and sensitivity to ischemia are in part due to the vasoprotective effects of estrogen in females.
- Estrogen is produced by P450 aromatase, which in addition to ovaries is expressed in multiple tissues, including vascular endothelium.
- We tested the hypothesis that sex differences in endothelial function and protection from ischemic dysfunction in brain are linked to endothelial aromatase.

Methods

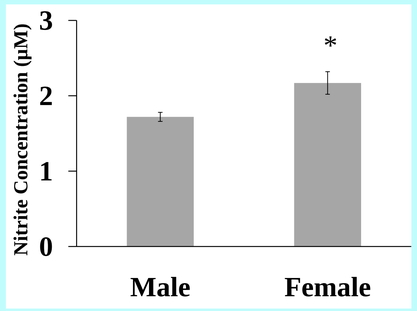
- Endothelial cells injury was assessed *in vitro* by lactate dehydrogenase (LDH) release after oxygen-glucose deprivation (OGD).
- Aromatase activity and expression were measured in isolated cerebral vessels and cultured endothelial cells from male and female mice.
- Aromatase immunoreactivity was localized in brain after MCA occlusion (MCAO) by immunohistochemistry.
- Endothelial function was assessed *in vivo* in wild-type (WT) and aromatase knockout (ArKO) mice.

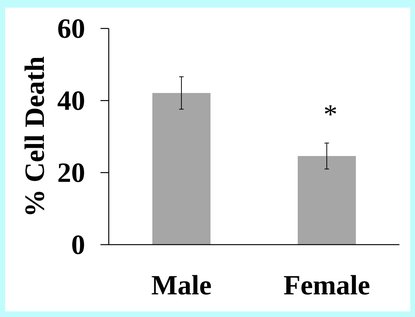
Results

Characterization of primary brain endothelial cell (EC) culture

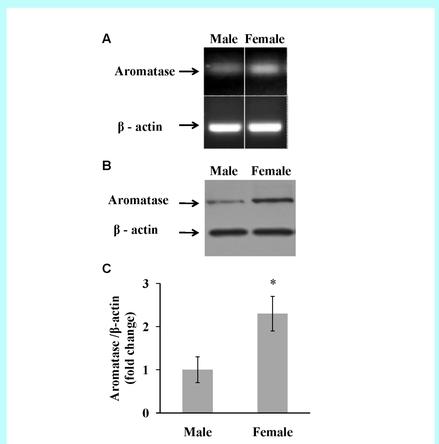


Endothelial cell function and response to ischemia *in vitro*

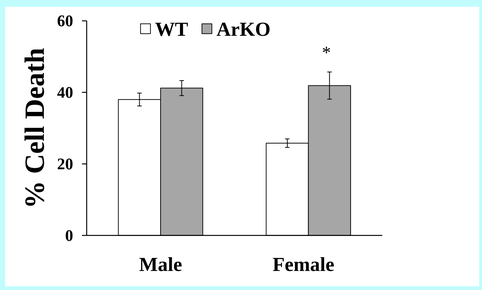




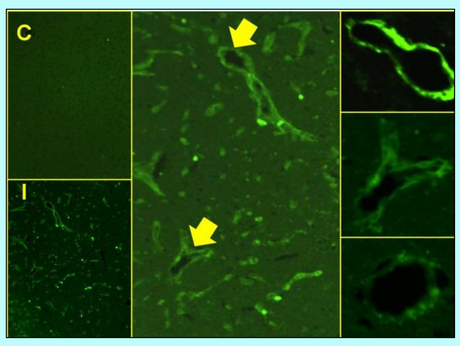
Sex differences in aromatase mRNA (A) and protein (B, C) expression in cultured endothelial cells



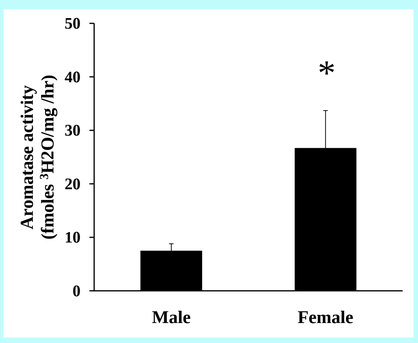
Sex differences in OGD-induced cell death is abolished in ArKO cells



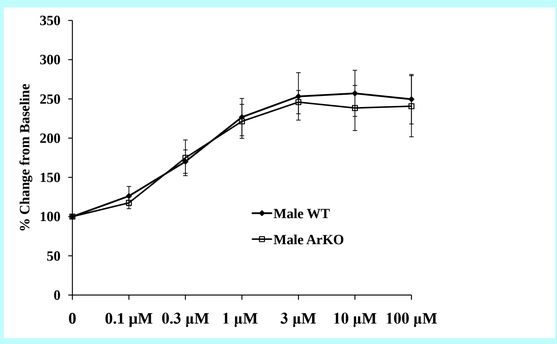
Immunofluorescent labeling of P450 aromatase in mouse brain sections in contralateral (C) and ischemic (I) cerebral cortex after MCAO

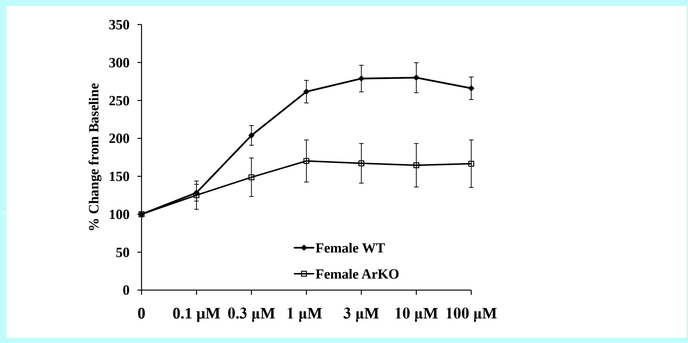


Aromatase activity was higher in female compared to male cerebral vessels after MCAO



Endothelium-dependent vasodilation in response to acetylcholine (ACh) in WT and ArKO cerebral vessels





Conclusion

- Female endothelial cells were more resistant to OGD than male endothelial cells. Aromatase gene deletion exacerbates ischemic injury in female, but not male endothelial cells, and the sex difference in response to OGD was not present in ArKO endothelial cells.
- Female endothelial cells express higher levels of aromatase mRNA and protein, and produce higher levels of NO in response to ACh stimulation than male endothelial cells.
- Aromatase immunoreactivity was localized in cerebrovascular endothelium. Mouse cerebral vessels express a functionally active aromatase, which is upregulated after ischemia in a sex-specific manner, with higher post-ischemic levels in female vs. male vessels.
- Aromatase gene deletion attenuates ACh dose-response curve in female, but not male cerebral vessels.
- We conclude that endothelial cell function and response to ischemia are sexually dimorphic, in part due to higher aromatase expression in female vs. male endothelial cells. The findings suggest that P450 aromatase plays a critical role in sex-specific endothelial cell function after cerebral ischemia.

References

1. Liu M, Hurn PD, Roselli CE and Alakyed NJ. Role Of P450 aromatase in sex-specific astrocytic cell death. Journal of Cerebral Blood Flow & Metabolism. 2007;27:135-41.
2. Liu M, Oyarzabal EA, Yang R, Murphy MJ, Hurn PD. A novel method for assessing sex specific and genotype specific response to injury in astrocyte culture. Journal of Neuroscience Methods. 2008; 171:214-217.