

Sex Differences in Response to Dihydrotestosterone and Flutamide in Astrocyte Cell Death

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Introduction

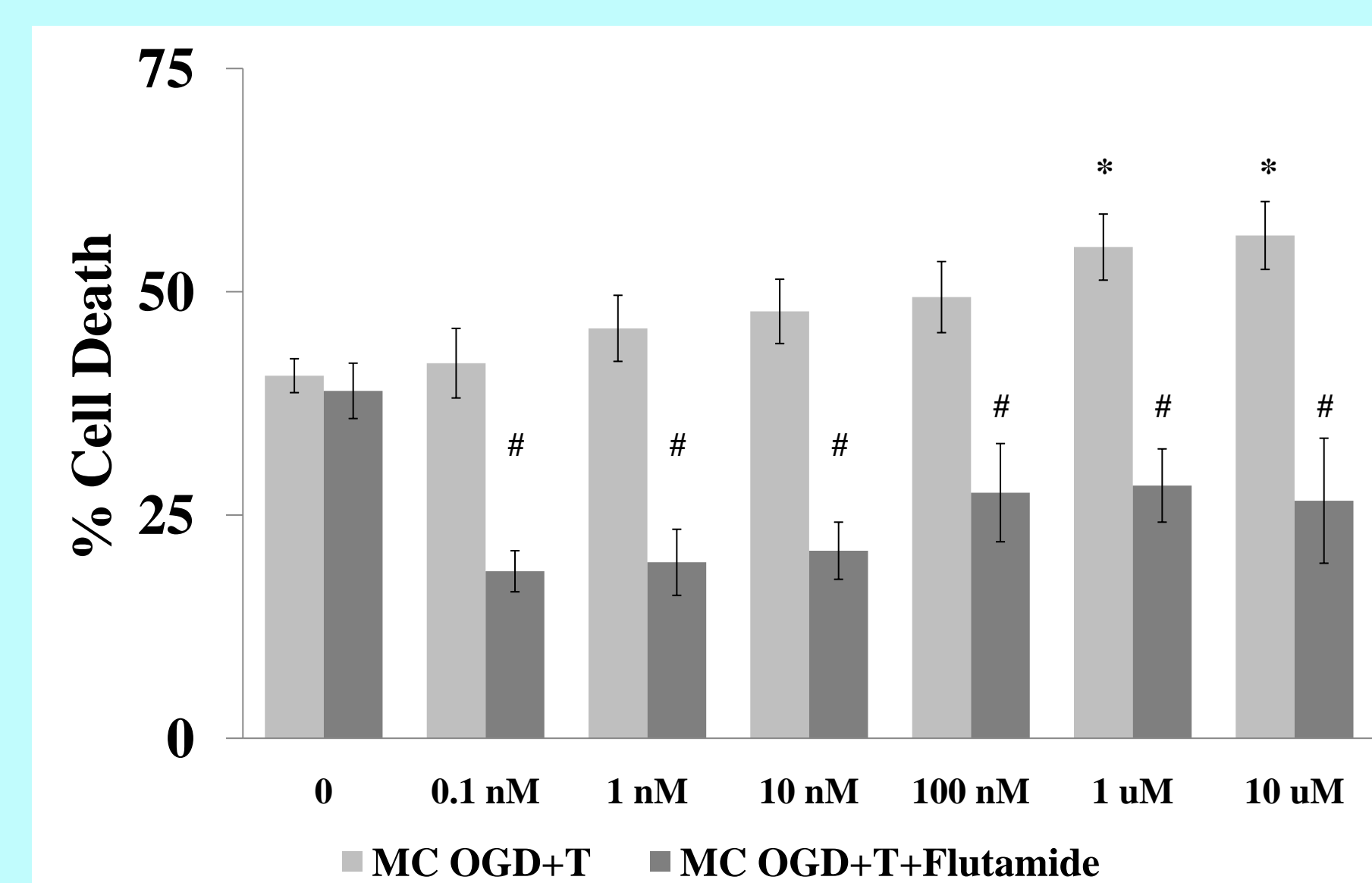
- Male sex is an acknowledged risk factor for stroke. Testosterone (T) can be converted to 17 β -estradiol via the aromatase, or metabolized to dihydrotestosterone (DHT) via the enzyme 5 α -reductase, a step that then does not permit aromatization to estradiol.
- We previously demonstrated that astrocytic cell death induced by oxygen-glucose deprivation (OGD) is sex-specific (1, 2). However, it is not clear if sex differences in response to T and DHT in astrocyte cell death contributes to the observed sex difference in response to ischemia.
- In the current study, we tested the hypothesis that male (XY) and female (XX) astrocytes respond differently to T and DHT.

Methods

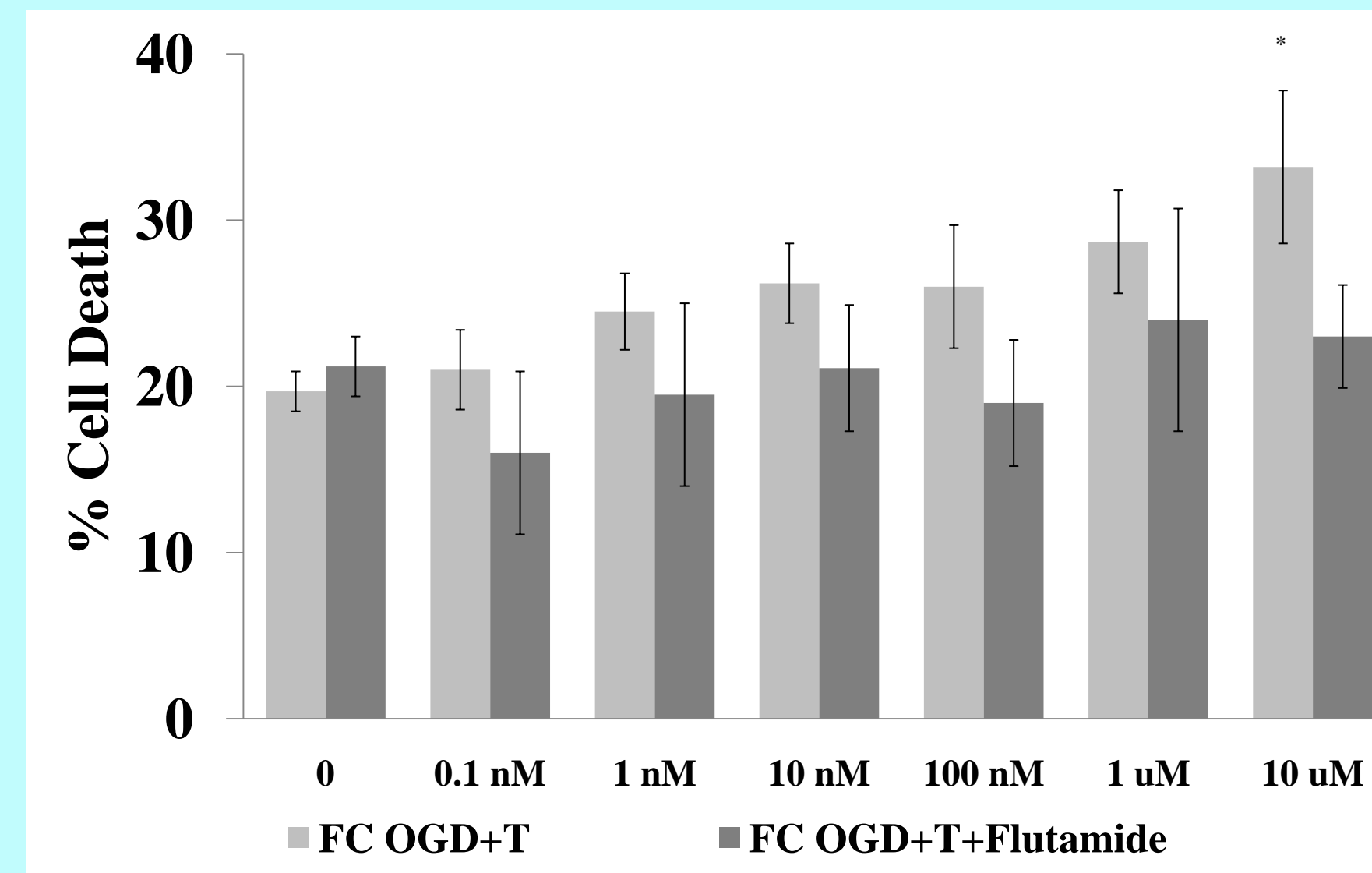
- Primary sex-specific cultured cortical astrocytes were prepared from 1-3-day old male and female rat pups separately and grown to confluency in steroid-free medium (1, 2).
- Confluent monolayers (10-14 days in vitro) were incubated in anoxia chamber in glucose-, serum-free medium for 6 hours OGD, and then returned to normoxia and glucose-containing medium for 24 hours.
- Cell death was induced by OGD alone, or in combination with T, DHT, or Flutamide (androgen receptor antagonist). These reagents were added 24 hours before OGD, and maintained during OGD and re-oxygenation.
- Cell death was measured at 24 hours after insult by lactate dehydrogenase (LDH) assay.
- Androgen Receptor, 5 α reductase 1 and 5 α reductase 2 mRNA expression was measured by real time quantitative PCR (qPCR).

Results

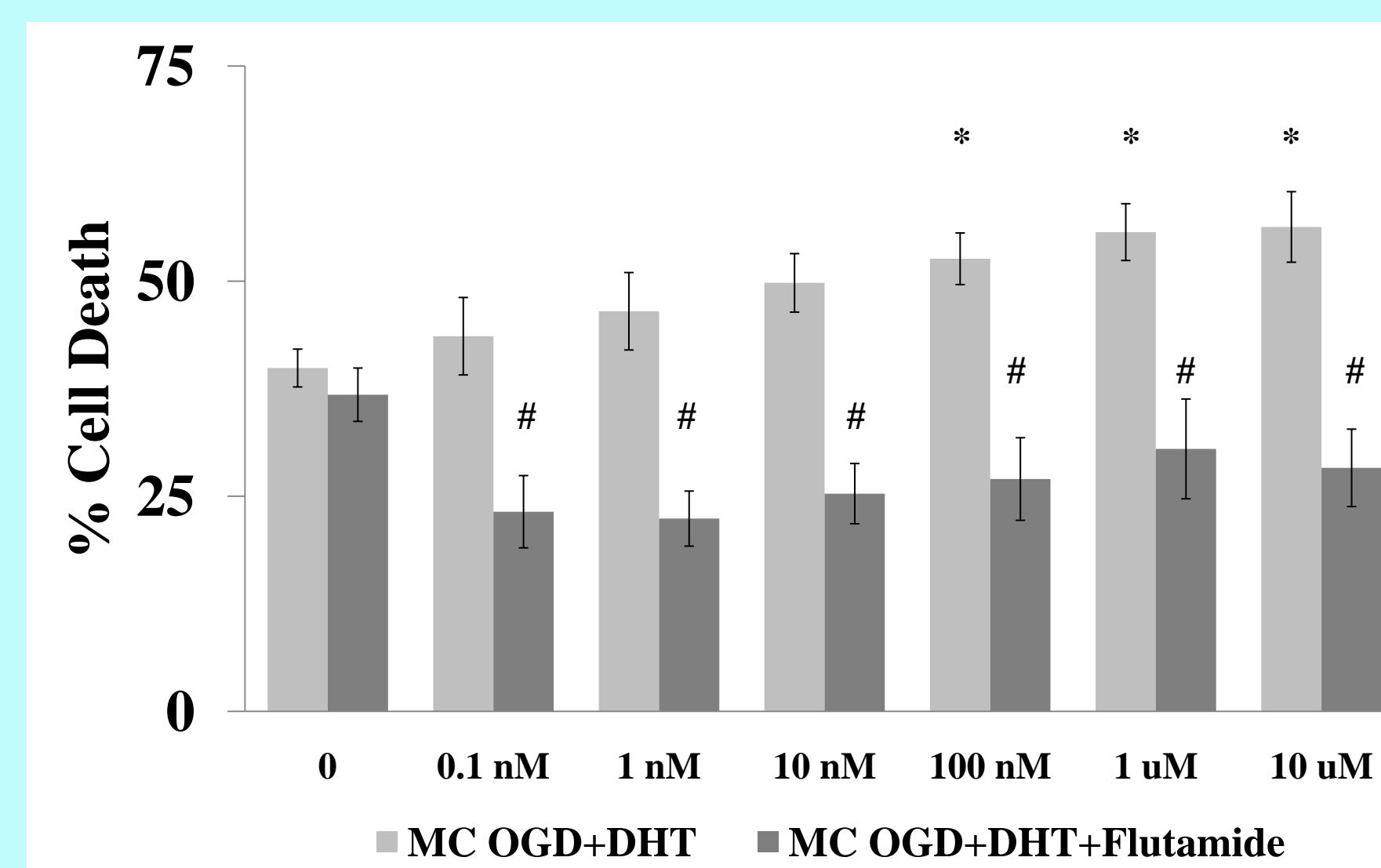
Dose Response of Testosterone on Male Astrocyte Cell Death



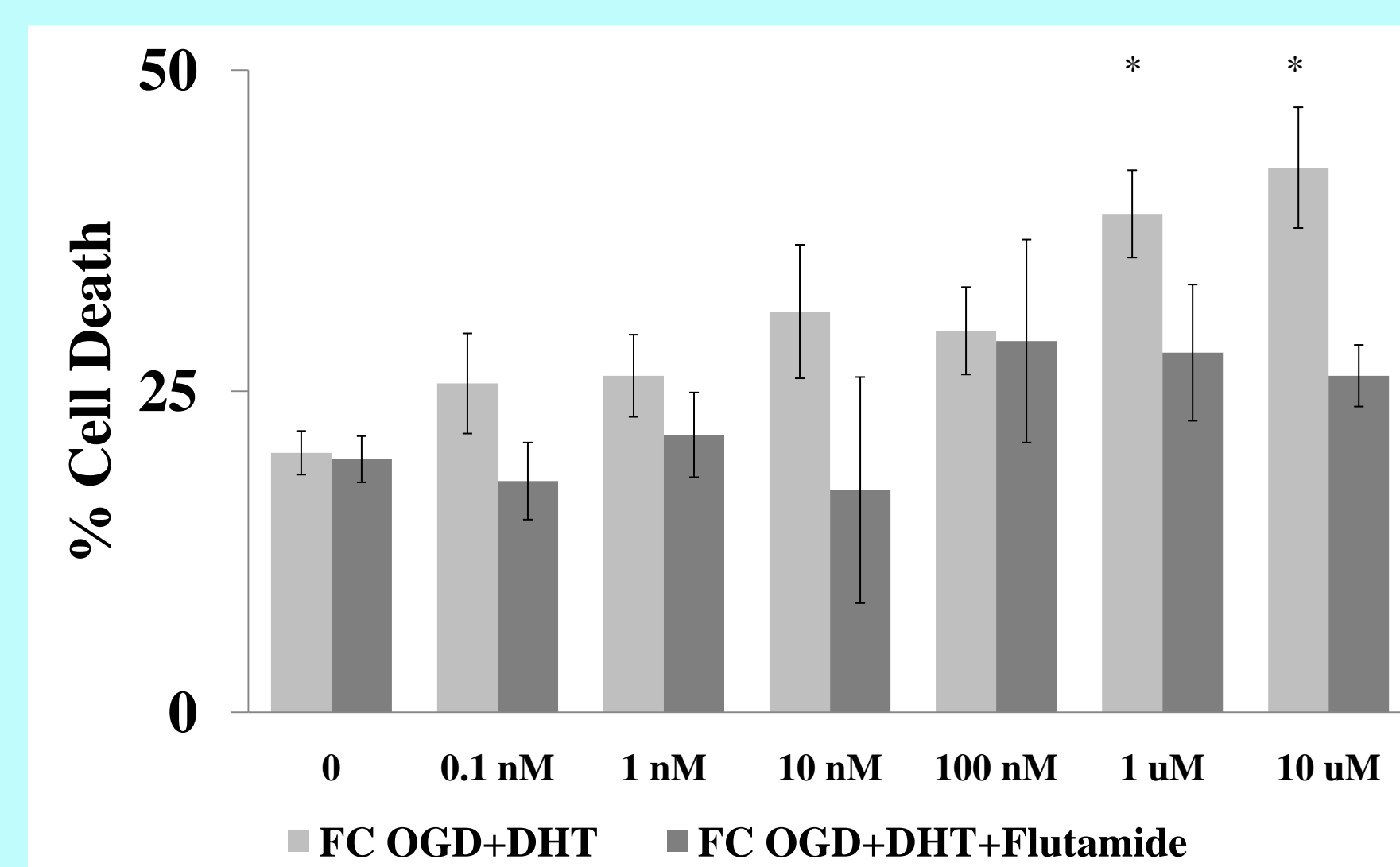
Dose Response of Testosterone on Female Astrocyte Cell Death



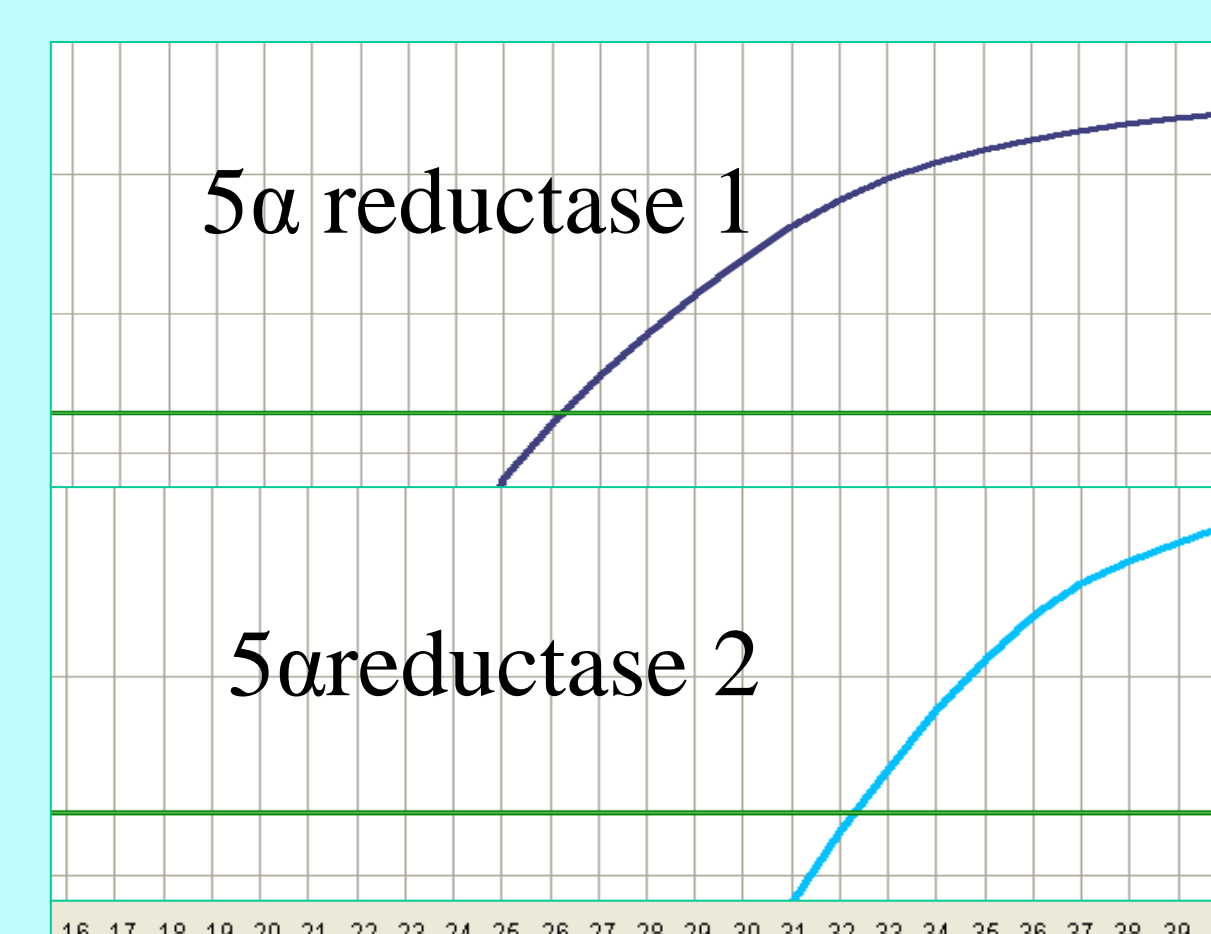
Dose Response of DHT on Male Astrocyte Cell Death



Dose Response of DHT on Female Astrocyte Cell Death

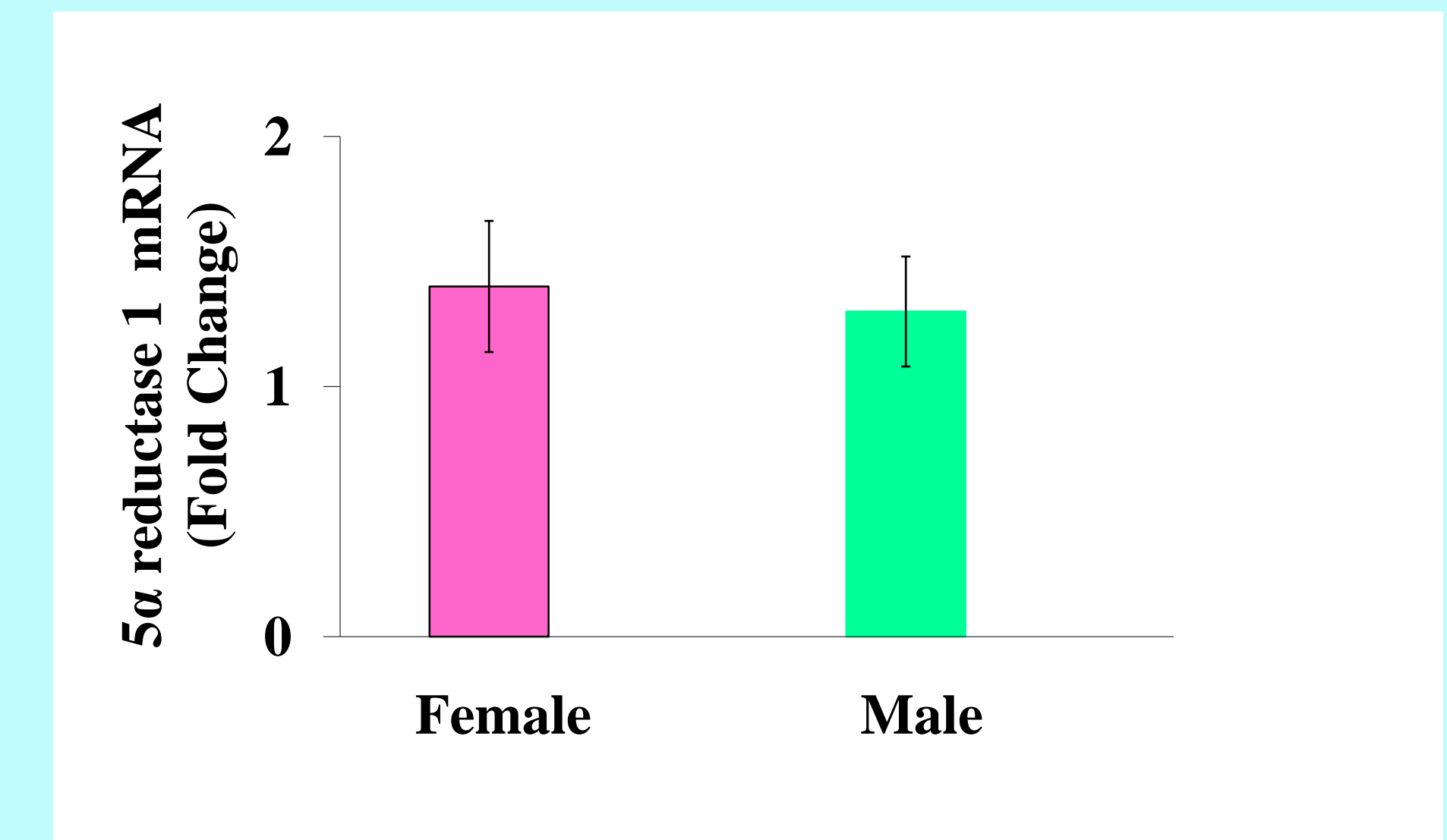


5 α reductase 1 and 5 α reductase 2 mRNA Expression in Astrocytes

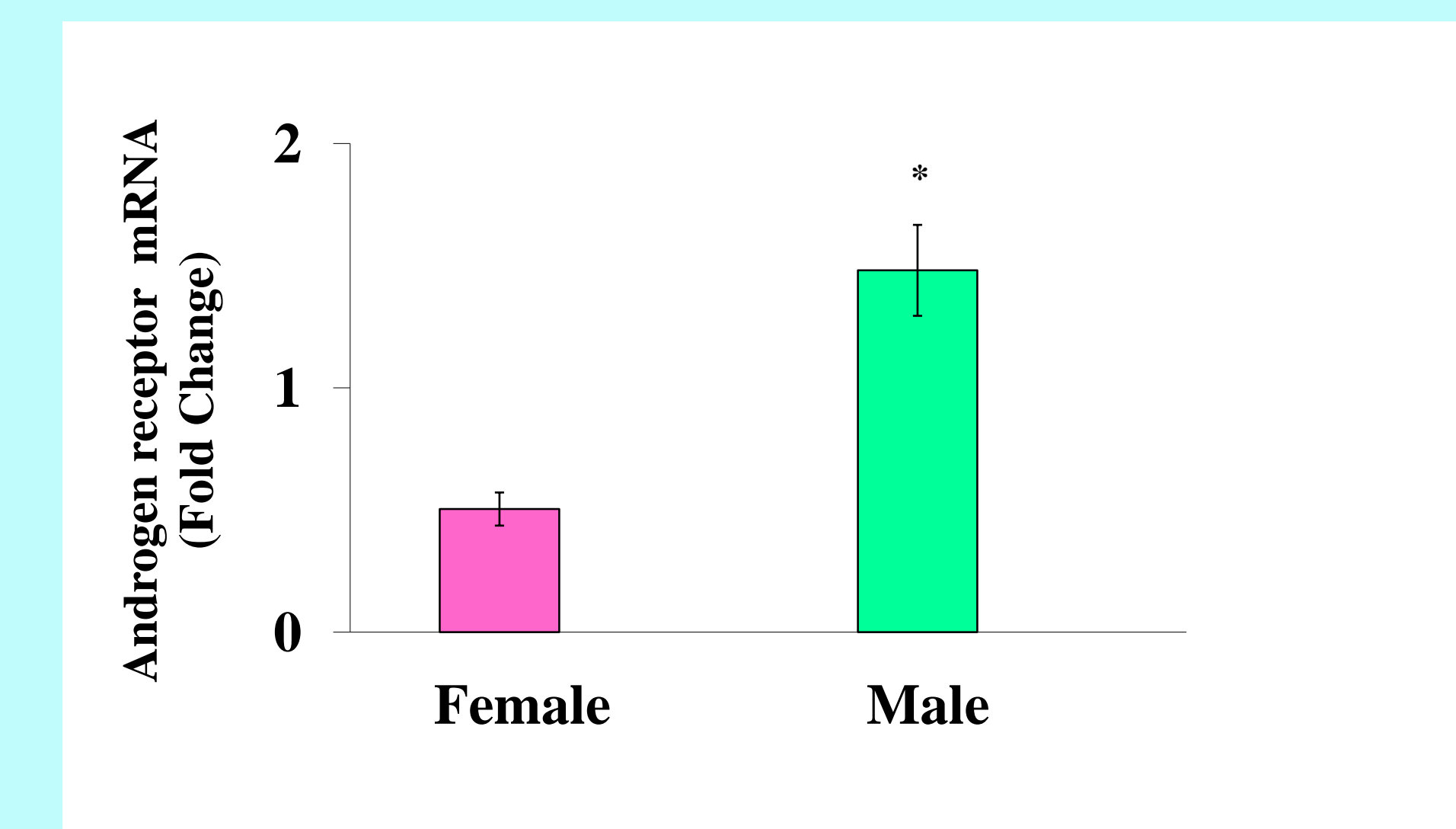


5 α reductase 2 mRNA expression level is much lower than 5 α reductase 1 in astrocytes, on the bottom of the detectable line. The Ct value is about 7 cycles late.

5 α reductase 1 mRNA Expression in Male vs. Female Astrocytes



Higher Androgen Receptor mRNA in Male vs. Female Astrocytes



Conclusion

- Female astrocytes are less sensitive to OGD alone, or in combination with T or DHT than male astrocyte. Androgen receptor antagonist, Flutamide, protect against T or DHT combined with OGD-induced cell death in male astrocytes but not in female astrocytes.
- 5 α reductase 1 mRNA expression was not different between male and female astrocytes.
- 5 α reductase 2 mRNA level was very low or undetectable both in male and female astrocytes.
- However, androgen receptor mRNA expression was sex difference in male vs. female astrocytes.
- We conclude that there are sex differences in ischemic sensitivity in female and male astrocytes, and male astrocytes (but not female) metabolize testosterone to DHT via the enzyme 5 alpha reductase, resulting in androgen receptor activation and enhanced cell death after OGD.

References

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- 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.