

Introduction: Male sex is a known risk factor in human stroke. Further, previous work suggests that androgens may have deleterious or beneficial effects on brain injury *in vivo*. It is well known that the biological activity of androgens occurs predominantly through binding to the intracellular androgen receptor (AR), which in turn acts as a transcription factor.

Aims: 1) To evaluate the role of AR overexpression in gonadally intact, male mice (AR-TG) treated with focal cerebral ischemia and 2) in an AR-transfected cell line (PC12 cells) treated with oxidative and apoptotic stressors. The ability of Testosterone and 5 α -dihydrotestosterone (DHT) to mitigate injury was also tested *in vitro*.

Results: We used a mouse model of AR overexpression, as previously published². AR-TG and wild type (AR-WT) mice were treated with middle cerebral artery occlusion (MCAO, 90 min) under isoflurane anesthesia and control of physiological variables. All animals were recovered for 24 hours of reperfusion. Outcome was determined by TTC histology. Infarction volume was reduced in cortex, striatum, and total hemisphere in AR-TG mice compared to WT animals.

To begin to elucidate the mechanisms of AR signaling and interaction with DHT, we studied an undifferentiated PC12 cell line that does not express AR (WT-PC12) and compared responses to cells stably transfected with cDNA constructs encoding full-length human AR controlled under the CMV promoter³ (AR-PC12). Cells were exposed to different paradigms of ischemic insult for 18 h and IC50s were determined for serum deprivation and oxidative stress using hydrogen peroxide (H₂O₂), Strauarospirine (STS) and Thapsigargin (Th)-induced apoptosis. Cell viability was determined by MTT staining. We observed that baseline cell viability increased in AR-PC12 when compared with WT-PC12 cells, in agreement with our observations in the AR-TG animal studies. As predicted, testosterone and DHT (10nM) treatment resulted in no protection against H₂O₂ or Th in WT-PC12 cells. In contrast, Testosterone, but not DHT significantly increased AR-PC12 viability by 30-300% after H₂O₂, Th, or STS exposure. **Conclusion:** Our results showed that 1) AR overexpression protects gonadally intact, male mice as compared to WT animals after the same ischemic insult, 2) AR-transfection protects AR-PC12 as compared to WT-PC12 to serum withdrawal, H₂O₂, STS and Th, and 3) Testosterone increases cell viability to these toxins only in AR-expressing cells. These data suggest a role for AR in modulating *in vivo* and *in vitro* brain injury. (1) J Cereb Blood Flow & Metab (2007), 1-10; (2) Endocrinol (2004) 145:3507; (3) PNAS 86: 327-332.

BACKGROUND

Male Androgens in Stroke

- Male sex known risk factor for stroke
- Decreased Testosterone (T) levels associated with larger infarct and poor outcome after acute stroke (Jeppesen et al., Arterioscler Thromb Vasc Biol 1996)
- T increases MCA occlusion lesion size in male rats (Cheng et al., JCBFM 2007)
- T accelerates functional recovery after stroke in male rats (Pan et al., Brain Res 2005)

Androgen Receptor (AR) in Brain

- AR distributed widely in the CNS (Finley et al., J Neurobiol 1999)
- AR upregulated after cerebral ischemia-reperfusion injury (Yang et al., J Neurobiol 2005)
- AR downregulated after castration (Lu et al., Endocrinology 1998)

Role of AR & Androgens on the Stroke Outcome remain Unclear

AIM 1

- To evaluate the role of AR overexpression in gonadally intact, male mice (AR-TG) treated with focal cerebral ischemia

Results (1)

METHODS

- In accordance with NIH guidelines and approved by the Institutional Animal Care and Use Committee at Oregon Health & Science University.
- Intact male mice 22-30g, sexually mature
- MCAO was performed under isoflurane anesthesia with laser-Doppler flowmetry.
- Head temperature (35.5 - 37.5°C) under left temporal muscle during MCAO and at reperfusion
- Infarct volume was determined by TTC staining at 24 h reperfusion



AR-TG Mice Study

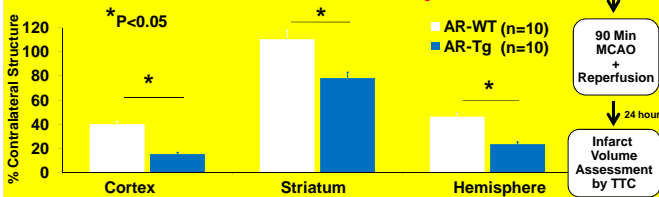


Figure 1. Infarct volumes were assessed at 24 h reperfusion after 90 min MCAO. AR-TG males showed smaller infarcts than AR-WT males.

AR-TG Neuronal Study

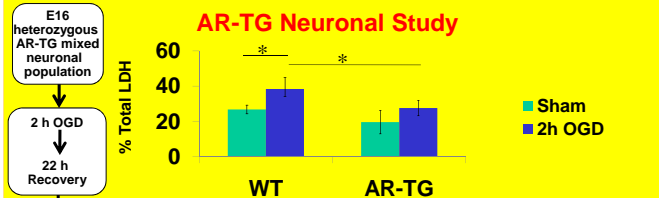


Figure 2. AR-overexpression in cortical neurons results in neuroprotection. Neuronal cultures from WT and heterozygous pups (AR-TG, 40-60% AR-TG) exposed to OGD (2h) and reoxygenation (22h).

AIM 2

- To evaluate AR-transfected cell line (AR-PC12 cells) response to injury and effects of T and DHT

AR-PC12 *in vitro* Study

Results (2)

METHODS

- PC12 cells were stably co-transfected with human sequence AR (AR-PC12) or Col2.3 β gal (Vector) and pRSneo and cultured in the presence of Geneticin.
- Cells were plated in 15% serum media for 24 h and grown in 2% serum media for 24 h. Finally, cells were exposed to different paradigms of ischemic insult for 18 h. Cell viability by MTT staining.

AR expression Protects PC12 from Serum Withdraw

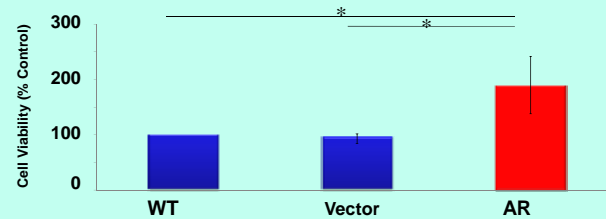


Figure 3. AR in PC12 is protective against serum deprivation. WT-PC12 (WT), PC12 transfected with vector (Vector) and AR-transfected PC12 (AR) were incubated in serum-free media for 18 h. Cell viability increased by 80% on AR-PC12 compared to WT and vector controls.

AR Expression Elicits Protection from H₂O₂-induced Oxidative Stress and STS- and Th-induced Apoptosis Stressors

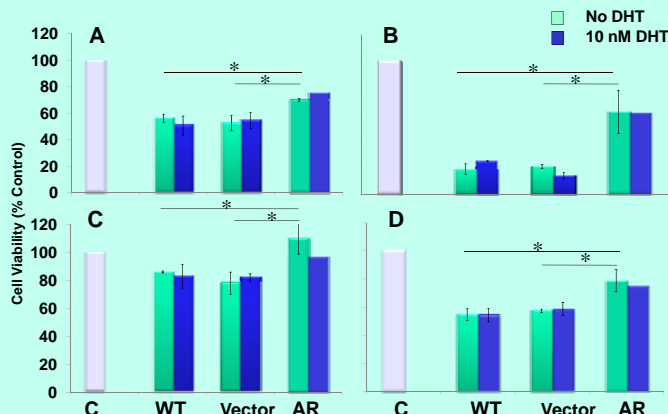


Figure 4. Protection against H₂O₂, STS and Th is AR dependent. Cultures incubated with 100 μ M (A), 200 μ M H₂O₂ (B), 8 nM STS (C) or 100 nM Th (D) with or without DHT for 18 h in serum-free media for 18 h. Cell viability was expressed as % of Control cells. Baseline cell viability increased in AR-PC12 (AR) when compared with WT-PC12 (WT) and vector-PC12 cells (Vector), in agreement with our observations in the AR-Tg animal studies (Figure 1). DHT treatment resulted in no extra protection against H₂O₂ in AR-PC12 cells.

Results (3)

Testosterone Protects AR-PC12 To Different Paradigms of Ischemic Insult

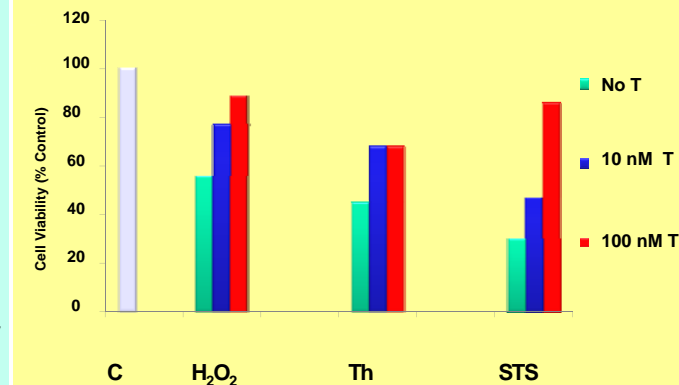


Figure 5. Testosterone protection is AR dependent. Cultures treated with 100 μ M H₂O₂ or 100 nM Thapsigargin (Th), or 40 nM STS for 18 h in serum-free media. Cell viability was expressed as % of vehicle control (C) cells. In the AR-expressing cells (AR), pretreatment with 10 and 100 nM testosterone significantly attenuated H₂O₂, Th and STS toxicity. Testosterone did not increase cell viability on WT cultures (data not shown). Data is representative of two different experiments.

CONCLUSIONS

- AR-overexpression protects AR-TG males from MCAO and cortical neurons from OGD
- AR-expression protects AR-PC12 from oxidative and apoptotic stressors
- These data suggest a role for AR in modulating *in vivo* and *in vitro* brain injury.

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