Androgen Receptor Expression in Human Coronary Vascular Smooth Muscle During Cytokine, Angiotensin II, or Hypoxic Exposure

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INTRODUCTION

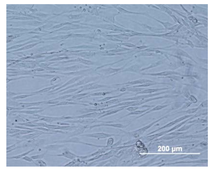
- Inflammation plays a key role in the pathogenesis of cerebro- and cardiovascular disease.
- Androgen receptor (AR) is present in human coronary artery vascular smooth muscle (VSM) (*Osterlund, et al. 2010*).
- In the **absence of inflammation**, androgens are pro-inflammatory in the vasculature; this response is **AR dependent** (*Razmara, et. al 2005; Gonzales, et. al 2009*).
- In the **presence of inflammation** the potent AR agonist, dihydrotestosterone (DHT), attenuates proinflammatory mediators following cytokine-, endotoxin-induced stimulation or hypoxia and glucose deprivation; this response is **AR independent** (*Osterlund, et. al 2010*).
- A functional splice-variant of AR, AR45 exists and has been shown to antagonize the DNA binding activity of AR in a dominant-negative interaction (*Ahrens-Fath, et. al 2005*).
- We investigated the effects pro-inflammatory stimulation (angiotensin II, hypoxia, and inerleukin-1 beta) on AR and AR45 expression in human VSM cells to determine if AR levels are altered in the presence of an inflammatory stimuli.

HYPOTHESIS

Pro-inflammatory mediators will reduce the expression of androgen receptor (AR) in primary human coronary artery vascular smooth muscle cells

METHODS

- Primary male human coronary artery VSM were cultured and studied at passages 6 to 9. Micrograph on right represents a typical cell population.



- PCR for SRY indicated cells were from a male donor.
- Cells were grown to 80% confluence, and exposed to the following treatment protocols in hormone free media:

	Exposure	Time
DHT	10nM, 50nM, or vehicle	chronic
IL-1β	5ng/ml or vehicle	12 hours
Ang II	100nM or vehicle	18 hours
Hypoxia	0.5% oxygen or vehicle	6 hours

- ***Western Blot:*** Human coronary and brain VSM cells were homogenized and assayed for protein content. Proteins were separated using SDS-PAGE, transferred to nitrocellulose membrane, and probed with anti-AR (N20) cat # sc-816 and anti-β actin or GAPDH primary antibodies from Santa Cruz Biotechnology.

RESULTS

AR is Present in Human Brain and Coronary VSM; Levels of AR Increased with Chronic DHT Treatment

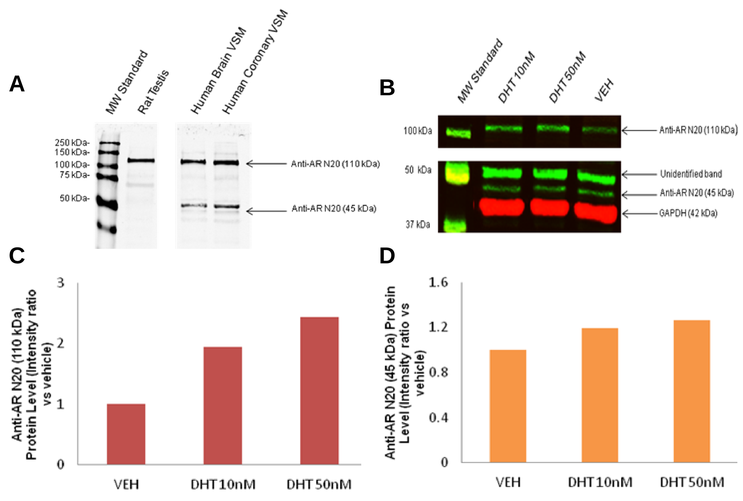


Figure 1. A: AR levels in rat testis lysate (positive control), human brain VSM, and human coronary VSM (HCVSM). Classical AR at 110 kDa is present in rat testis and human VSM. The band at 45 kDa is present in HCVSM and human brain VSM and is not present in rat testis lysate. B: Western blot of AR, putative AR45, and GAPDH in HCVSM exposed to DHT (10 and 50 nM). In HCVSM, AR (panel C) and putative AR45 (panel D) are increased following DHT.

Cytokine Treatment Decreased AR and AR45 Levels

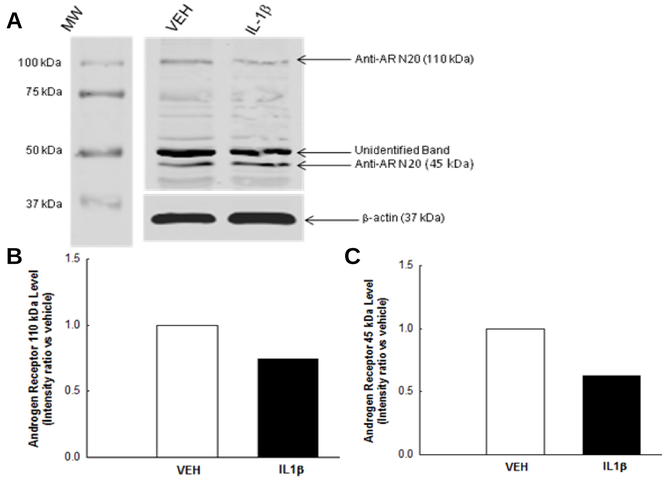


Figure 2. A: Representative blot. IL-1β response on AR (panel B) and AR45 (panel C) levels in HCVSM. Cytokine treatment decreased AR by 26% and putative AR45 by 38%, n=1 compared to vehicle (VEH); n=2

Angiotensin II Decreased AR Levels and did not alter AR45 Levels

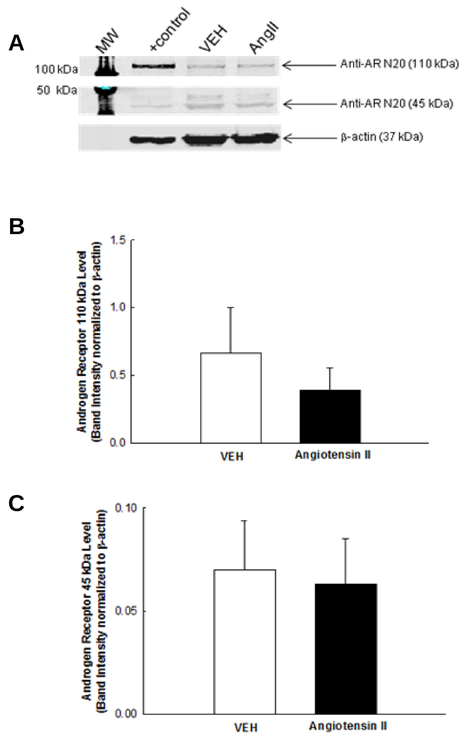


Figure 3. A: Representative blot. Angiotensin II (n=3) decreased AR by 41% (panel B; p = 0.026) and putative AR45 by 10% (panel C; p = 0.95) compared to vehicle (VEH) (n=3). Angiotensin II did not effect levels of putative AR45. (+ control = rat testis lysate)

Hypoxia did not alter AR in HCVSM

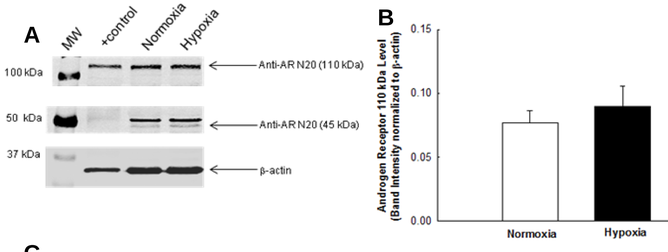


Figure 4. A: Representative blot. Although there was a trend for an increase in AR by 18% compared to normoxia (panel B; n=3, p=0.52), the difference was not statistically significant. Levels for the AR45-like band were increased by 16% compared to normoxic (vehicle) treated cells (panel C; n=3, p=0.078). (+ control = rat testis lysate)

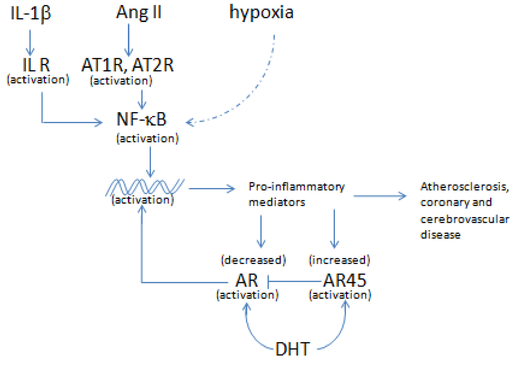
SUMMARY

- AR and AR45 are present in HCVSM and DHT increased receptor levels (**Figure 1**).
- AR and AR45 are reduced by the pro-inflammatory cytokine IL-1β (**Figure 2**).
- AR protein levels are decreased by Angiotensin II, however AR45 levels were not altered (**Figure 3**).
- Hypoxia did not effect AR levels, but may cause an increase in AR45 (**Figure 4**).

INTERPRETATION

Pro-inflammatory mediators reduce the expression of AR. IL-1β directly activates the pro-inflammatory transcription factor NF-κB by acting on the IL1 receptor. Ang II has been shown to activate NF-κB directly by acting on AT1 and AT2 receptors (*Ruiz-Ortega, et. al 2000*). Hypoxia may be unable to directly activate NF-κB, therefore did not change AR levels.

AR45 stimulation has been shown to inhibit transcriptional activity of AR. AR45 has also been shown to be expressed in cardiac tissue (*Ahrens-Fath, et. al, 2005*) and to be highly conserved throughout mammalian evolution (*Weiss, et. al, 2007*). Decreasing ratio of AR to AR45 in inflammatory states may be one way by which DHT attenuates IL-1β induced inflammation in HCVSM. Summary diagram (right hand side).



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