Ascorbic Acid Decreases the Binding Affinity of the AT\textsubscript{1} Receptor for Angiotensin II

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What is Ascorbic Acid?

- Ascorbic Acid is more commonly known as Vitamin C.
Angiotensin II
Background

- Ascorbic acid
  - Essential nutrient
  - Antioxidant (Neutralizes ROS)
  - Claimed by many to have beneficial effects on chronic cardiovascular diseases
  - “Several studies have shown that the plasma concentration of ascorbic acid is inversely related to blood pressure,\(^5\)-\(^9\) while an increase intake of ascorbic acid has been associated with lower blood pressure.\(^{10-13}\)” (67)
Background (cont’d)

- Angiotensin Pathway

Water and salt retention. Effective circulating volume increases. Perfusion of the juxtaglomerular apparatus increases.
Physiological Effects of Ang II

- **Cardiovascular**
  - Vasoconstrictor of Arteries and veins
  - Increases blood pressure

- **Neural**
  - Increases secretion of ADH in the posterior pituitary
  - Increases secretion of ACTH in the anterior pituitary

- **Adrenal**
  - Causes release of aldosterone

- **Renal**
  - Increases glomerular filtration rate by increasing renal perfusion via efferent renal arteriole constriction.
Materials

- HEK-293 used to express the $\text{AT}_1$ receptor for Ang II by transfection.
- Smooth muscle contraction studies were performed using rabbit aorta strips that endogenously express the $\text{AT}_1$ receptor.
What are HEK-293 cells?

- HEK stands for Human Embryonic Kidney cells
  - Easily grown
  - Transfect readily
  - Used throughout biological research
  - Used in the biotechnology industry to produce therapeutic proteins and viruses for gene therapy.

- Used essentially as a test tube with a membrane

- Not a good model for normal cell, cancer cells, or any other kind of cell that is a fundamental object of research
HEK-293 AT$_1$ Reactivity to Ang II

Methods

1. HEK-293 transfection and Ang II binding
   - HEK-293 cultured and transfected with DNA to express AT₁ receptors
   - Cells stably expressing AT₁ receptors were incubated in radioactive $^{125}$I-Ang II with and without ascorbic acid

2. Video imaging of intracellular Ca$^{2+}$ oscillations
   - Ca$^{2+}$ assays were conducted on HEK-293 expressing AT₁
Methods (cont’d)

3. N.Z. White Rabbit aorta Ang II contractile response

- Helical strips of rabbit aorta were collected from anesthetized animals.
- Stretched sections of aortic tissue to tension of 2 g and recorded contractile responses.
Results

- Figure 1a shows that ascorbic acid decreased the specific binding of $^{125}$I-Ang II to AT$_1$.
- Maximum inhibitory effect achieved with 100 µmol/L ascorbic acid.
Results (cont’d)

- AT₁, like other GPCRs, lose affinity for its ligands when the G-Protein becomes uncoupled from its receptor.
- G-Protein uncoupling reagents are able to decrease the Ang II affinity to AT₁.
- This indicates that ascorbic acid does not inhibit binding by G-Protein uncoupling.
- In parallel experiments, ascorbic acid had no effect on AT₂ receptor affinity for Ang II.
- Once Ang II was added to aortic tissue, contraction occurred.
- Ascorbic acid partially decreased contractile response, resulting in decreased tension (Figure 4a).
Discussion

- Ascorbic acid specifically decreases the binding affinity of the $\text{AT}_1$ receptor.
- $\text{AT}_1$ expression on cellular surface is unaffected by the presence of ascorbic acid.
- Further studies should elucidate the exact mechanism of ascorbic acid interaction with $\text{AT}_1$.
- In vitro experiments with rabbit aortic tissue showed the effect of ascorbic acid in animal tissue on hypertension.