# #559: Mechanisms involved in nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Nox)-derived reactive oxygen species (ROS) modulation of muscle function in human bladders

Nagat Frara [1] *, Dania Giaddui [1], Alan S. Braverman [1], Mary F. Barbe [1], Changhao Wu [2] and Michael R. Ruggieri, Sr.[1]

[1]Center for Translational Medicine, Lewis Katz School of Medicine, Temple University, Philadelphia, PA, USA, and
[2]Department of Biochemistry and Physiology, Faculty of Health and Medical Sciences, University of Surrey, Guildford, UK

## Introduction

Generation of reactive oxygen species (ROS) by the enzyme nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Nox) is a highly regulated process that constitutes one of the main redox signaling components (1). Excessive ROS production may cause oxidative damage to tissue and organ. Although, the role of redox signaling in different pathological processes has been of intense interest (2), its importance in bladder pathology remains unexplored.
**Hypothesis/aims of study:** Our aim was to use *in vitro* muscle strip contractility studies to explore the physiological role of ROS/Nox in regulating bladder function in humans.

## Methods and Materials

- Mucosa-denuded bladder muscle strips were obtained from human organ transplant donors. Strips from 3 males and 2 females were mounted in muscle baths.
- Trains of electrical field stimulation (EFS) of 1 ms pulse duration, 12 V, 8 Hz at 90 s intervals were applied to each strip for about 20 minutes.
- Subsets of strips were incubated with ROS/Nox agonists or antagonists for 20 minutes in continued trains of EFS.
- Same subsets of strips that were treated with antagonists were retreated with agonists and their responses were detected.
- All responses are expressed in milli Newtons (mN). Data is presented as mean ± 95% CI.

## Results

### Effect of 100μM $H_2O_2$ on EFS-induced contractions

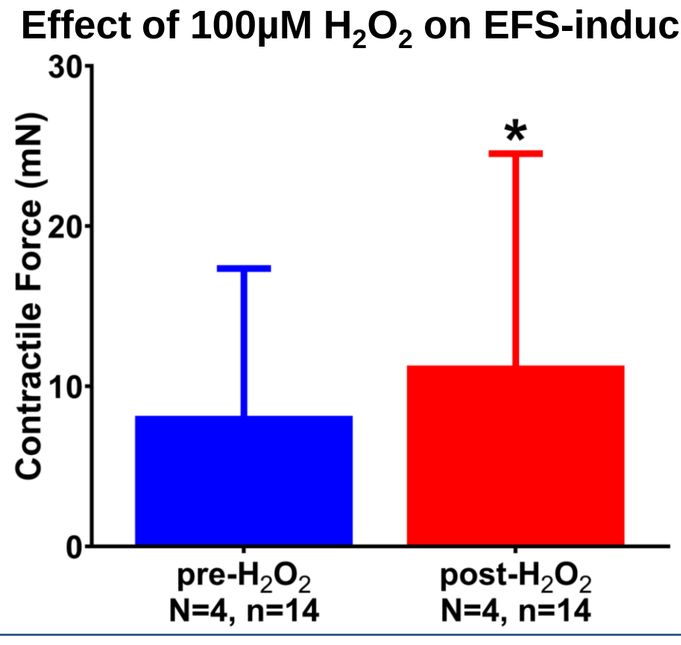


**Figure 1.** Exogenous ROS hydrogen peroxide ($H_2O_2$) enhances EFS-induced muscle contractions (**N=4, n=14**). *: $p < 0.05$.

### 100μM $H_2O_2$–induced muscle strip contraction

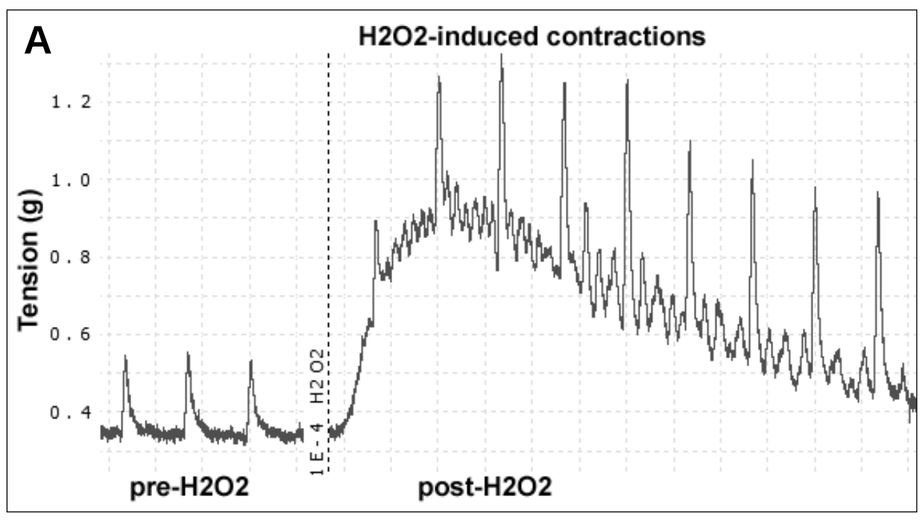


**Figure 2. A)** Representative image of muscle strip contraction after the addition of $H_2O_2$ to the muscle bath.
**B)** $H_2O_2$-induced muscle strip contraction in human bladders (**N=4, n=14**).

### Effect of 100μM apocynin on EFS-induced contractions

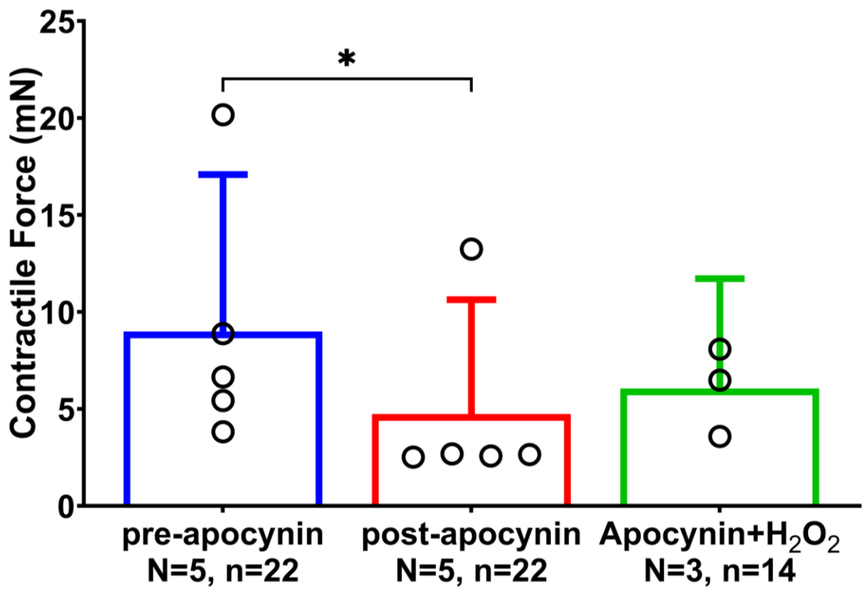


**Figure 3.** ROS generating enzyme Nox inhibitor (**apocynin)** attenuates intrinsic muscle strip activity (**N=3-5, n=14-22**). *: $p < 0.05$.

## Results

### Effect of 1μM angiotensin II on EFS-induced contractions

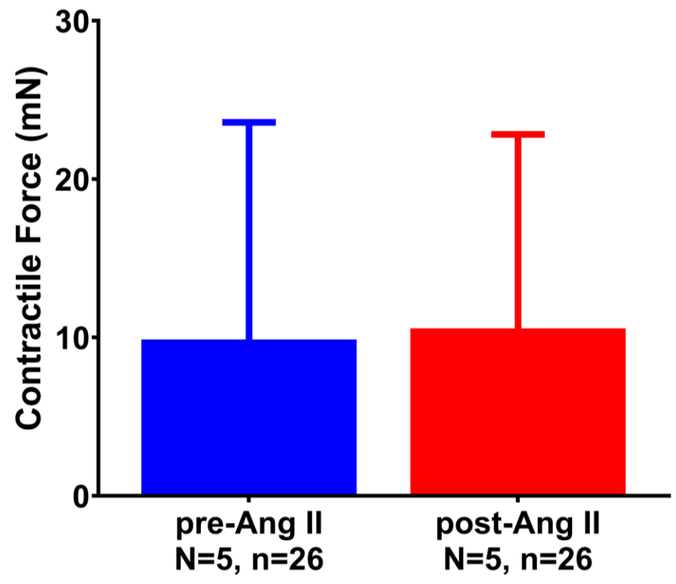


**Figure 4.** Application of a key Nox activator and a pro-inflammatory peptide **angiotensin II (Ang II**) has no effect on EFS-induced smooth muscle contractions (**N=5, n=26**).

### 1μM Angiotensin II–induced muscle strip contraction

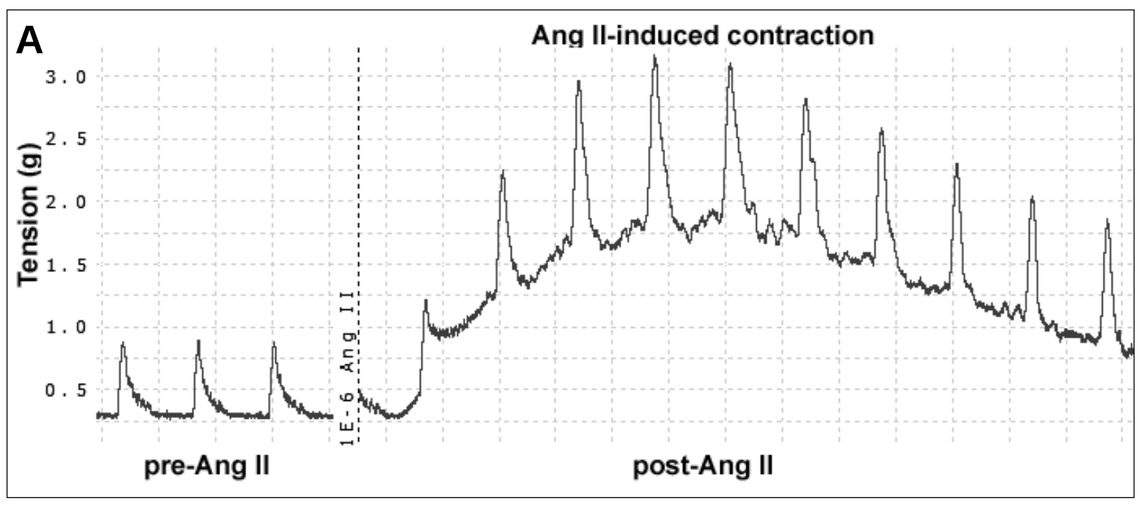


**Figure 5. A)** Representative image shows that application of **1μM angiotensin II** enhances smooth muscle strip contraction.
**B) angiotensin II**-induced muscle strip contraction in human bladders (**N=5, n=26**).

### Effect of 10μM ZD7155 on EFS-induced contractions

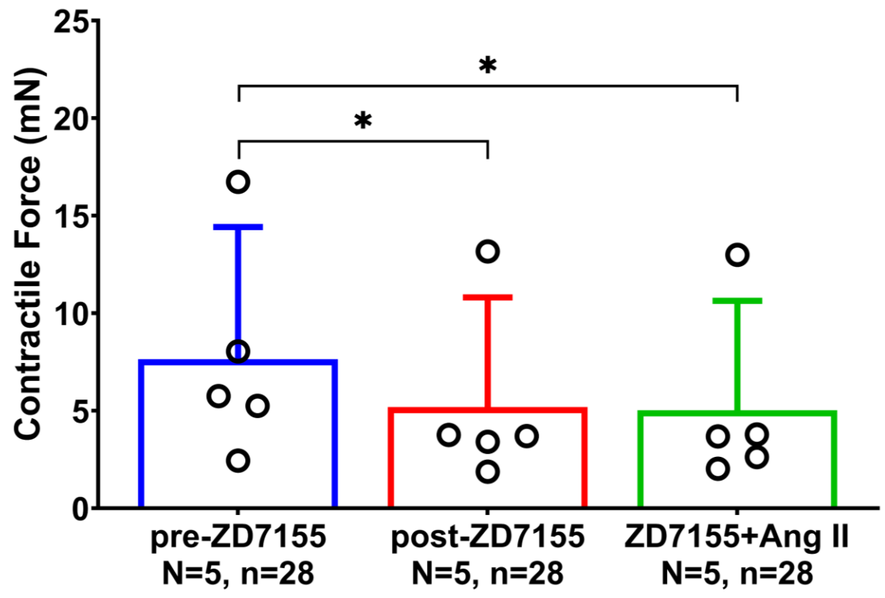


**Figure 6.** Blockade with AT1 receptor-specific antagonist (**ZD7155**) inhibited EFS-induced activity (**N=5, n=28**). * : $p < 0.05$.

## Discussion

- The enhancement of EFS-evoked contractions by H2O2 and the inhibition of these contractions by the Nox inhibitor apocynin demonstrates the functional relevance of ROS in regulating human bladder smooth muscle activity and suggests that endogenous Nox-derived ROS regulates smooth muscle function.
- The augmentation of contractions by angiotensin II suggests that activation of Nox via a receptor by its ligand can also enhance smooth muscle activity and that the effect of angiotensin II is mediated by AT1, which was further supported by the inhibitory effect of the selective antagonist ZD7155.

## Conclusions

- Collectively, these data provide evidence for the functional significance of Nox-derived ROS in human bladder and that ROS can modulate bladder function without exogenous stimuli.
- Since, inflammation is an important mechanism associated with oxidative damage, the effects of angiotensin II on bladder smooth muscle function may have significant pathologic implications.

## References

1- Burgoyne J.R. et al. Circulation Research. 2012;111(8):1091-106. doi: 10.1161/CIRCRESAHA.111.255216.
2- D'Autréaux B. & Toledano M.B. 2007. Nat. Rev. Mol. Cell Biol. 2007; 8:813–824. doi: 10.1038

## Acknowledgements

This research was supported by a grant from the NIH. NIA 1R01AG049321-01A1 to CW and MRR