#655: Reactive oxygen species (ROS) and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Nox) enhance neurogenic-induced muscle contractions in human and dog bladders

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Introduction

Oxidative stress has been directly linked to urinary bladder pathologies [1,2]. However, roles of redox signaling in bladder function is still under investigation. Therefore, we aimed to explore the physiological role of reactive oxygen species (ROS) and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Nox) in regulating muscle function, and to examine mechanisms of Nox activation, in normal bladders from humans and dogs.

Methods and Materials

- Mucosa-denuded muscle strips obtained from bladders of 7 human organ donors and 4 normal dogs were mounted in muscle baths.
- Trains of electrical field stimulation (EFS) of 1 ms pulse duration, 12 V, 8 Hz applied to each strip for 20 minutes at 90-second intervals.
- Subsets of strips were incubated with hydrogen peroxide (H_2O_2), angiotensin II (Ang II; Nox activator), apocynin (inhibitor of Noxs and ROS scavenger), or ZD7155 (specific inhibitor of angiotensin type 1 (AT1) receptor) for 20 minutes in continued EFS trains.
- Strip treated with inhibitors were then treated with H_2O_2 or Ang II, without washout of prior treatment(s).
- Contractions are expressed in milli Newtons (mN). N = number of specimens, n = number of strips. Data is presented as mean ± 95% CI.
- Superoxide levels were measured over time using lucigenin-enhanced chemiluminescence in adjacent segments of dog bladder muscle tissue. High increase in ROS was triggered by 100µM NADPH. Superoxide was scavenged by 20 mM Tiron.

Results

Exogenous ROS, hydrogen peroxide (H_2O_2) enhanced EFS-induced strip contractions in human & dog bladders

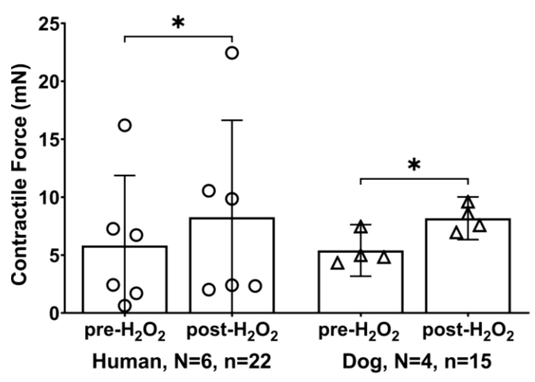

Figure 1. Electrical field stimulation (EFS)-induced strip contractions in response to exogenous **hydrogen peroxide (H_2O_2, 100µM).** *: $p < 0.05$, comparing pre- vs post-H_2O_2 treatment.

100µM H_2O_2–induced direct muscle strip contraction

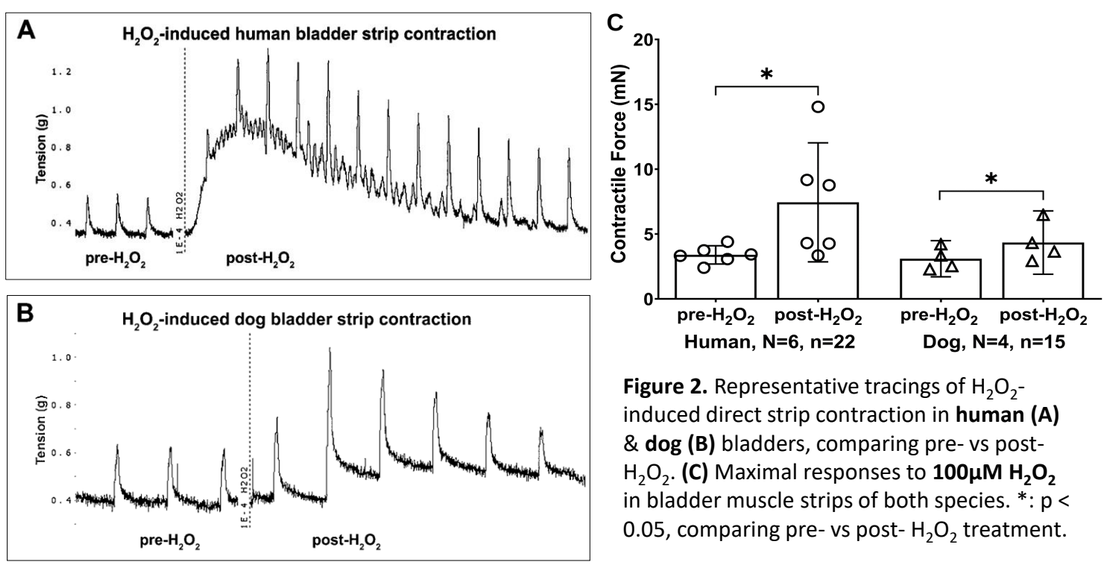

Figure 2. Representative tracings of H_2O_2-induced direct strip contraction in **human (A)** & **dog (B)** bladders, comparing pre- vs post-H_2O_2. **(C)** Maximal responses to **100µM H_2O_2** in bladder muscle strips of both species. *: $p < 0.05$, comparing pre- vs post- H_2O_2 treatment.

Nox inhibitor and ROS scavenger, apocynin, attenuated EFS-induced strip contractions in human & dog bladders

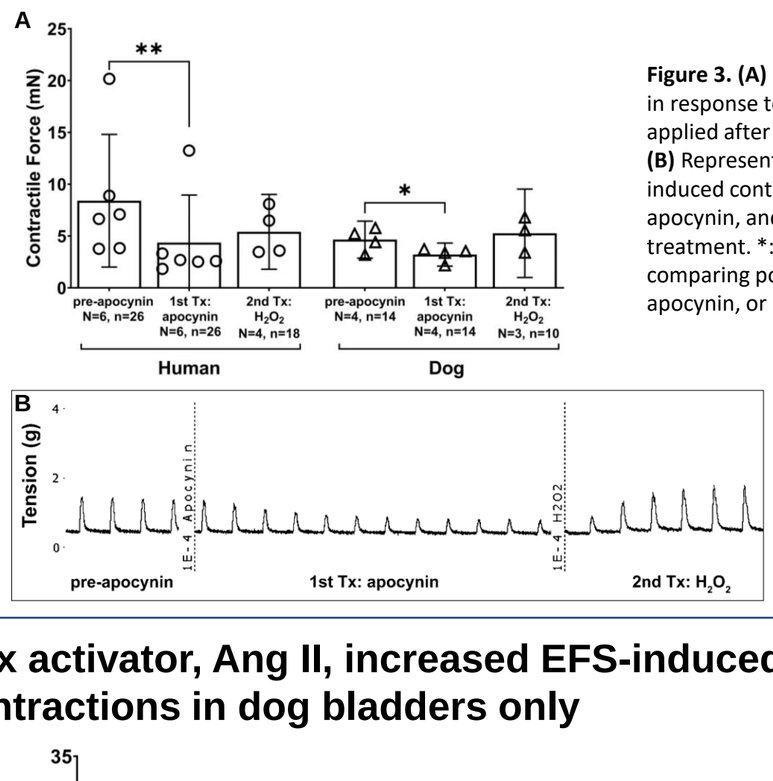

Figure 3. (A) EFS-induced contractions in response to **H_2O_2 (100µM, 2nd Tx)** applied after **apocynin (100µM, 1st Tx). (B)** Representative tracing of EFS-induced contractions in response to apocynin, and then H_2O_2. Tx = treatment. *: $p < 0.05$ and **: $p < 0.01$, comparing post-apocynin vs either pre-apocynin, or H_2O_2 treatments.

Nox activator, Ang II, increased EFS-induced muscle strip contractions in dog bladders only

Figure 4. EFS-induced strip contractions in response to **angiotensin II (Ang II, 1µM)**. *: $p < 0.05$, comparing pre- vs post-Ang II treatment.

Results

Ang II increased direct muscle strip contraction significantly in human bladders

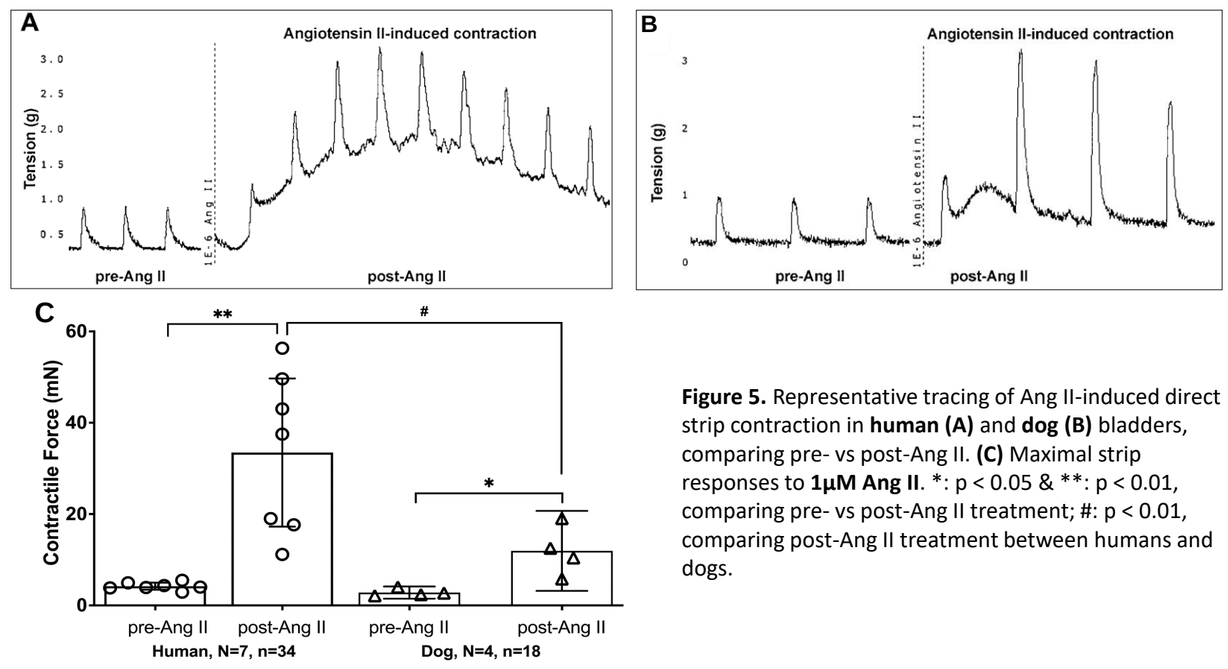

Figure 5. Representative tracing of Ang II-induced direct strip contraction in **human (A)** and **dog (B)** bladders, comparing pre- vs post-Ang II. **(C)** Maximal strip responses to **1µM Ang II**. *: $p < 0.05$ & **: $p < 0.01$, comparing pre- vs post-Ang II treatment; #: $p < 0.01$, comparing post-Ang II treatment between humans and dogs.

Ang II treatment after apocynin treatment enhanced EFS-induced contractions in both species

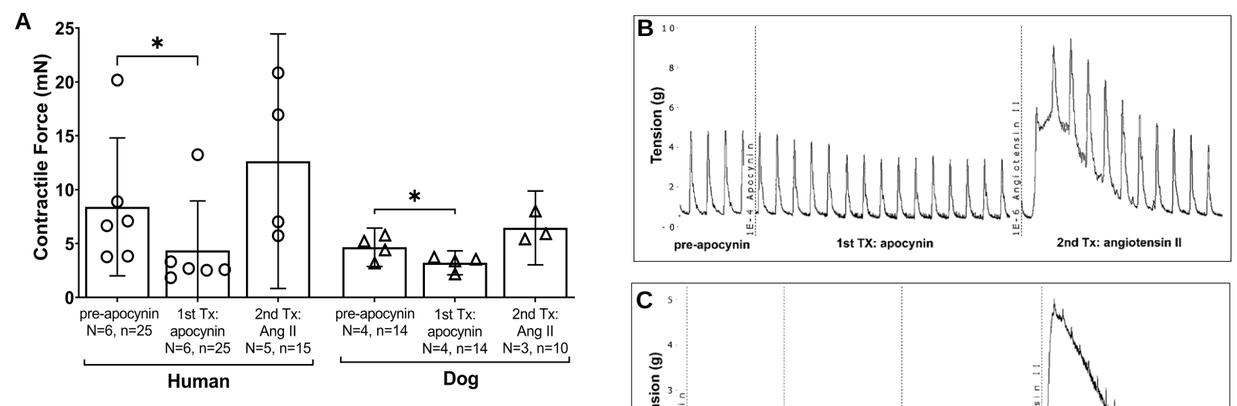

Figure 6. (A) EFS-induced contractions in response to **Ang II (1µM, 2nd Tx)** applied after **apocynin (100µM, 1st Tx).** Representative tracing of EFS-induced contractions in response to apocynin (1st Tx), and then Ang II (2nd Tx) **(B)** or apocynin, then H_2O_2, and then Ang II (3rd Tx) **(C)** . *: $p < 0.05$, comparing post-apocynin to pre-apocynin, or to Ang II treatments.

The AT1 receptor-specific inhibitor, ZD7155, attenuated EFS-induced contractions

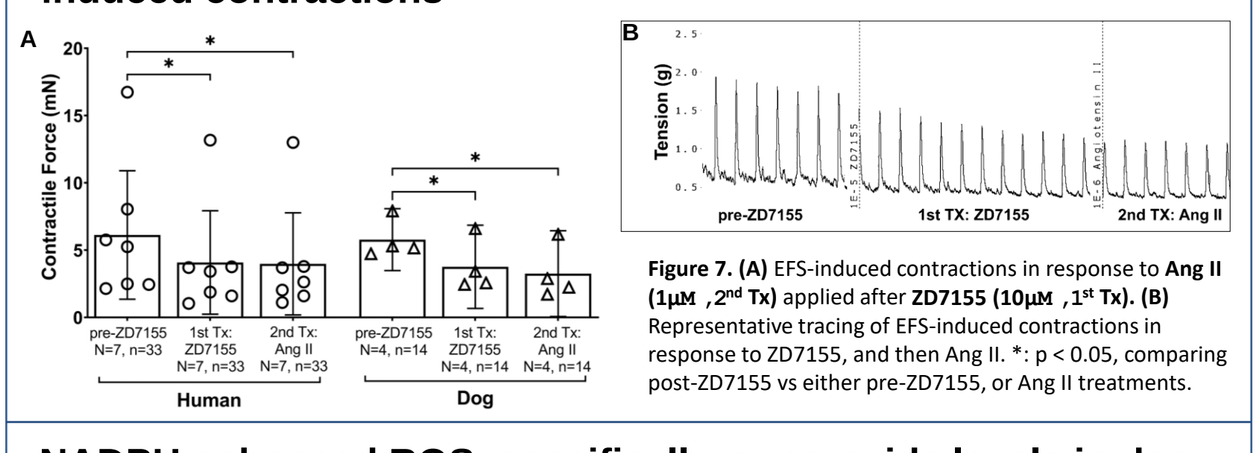

Figure 7. (A) EFS-induced contractions in response to **Ang II (1µM, 2nd Tx)** applied after **ZD7155 (10µM, 1st Tx). (B)** Representative tracing of EFS-induced contractions in response to ZD7155, and then Ang II. *: $p < 0.05$, comparing post-ZD7155 vs either pre-ZD7155, or Ang II treatments.

NADPH enhanced ROS, specifically, superoxide levels in dog bladder smooth muscle

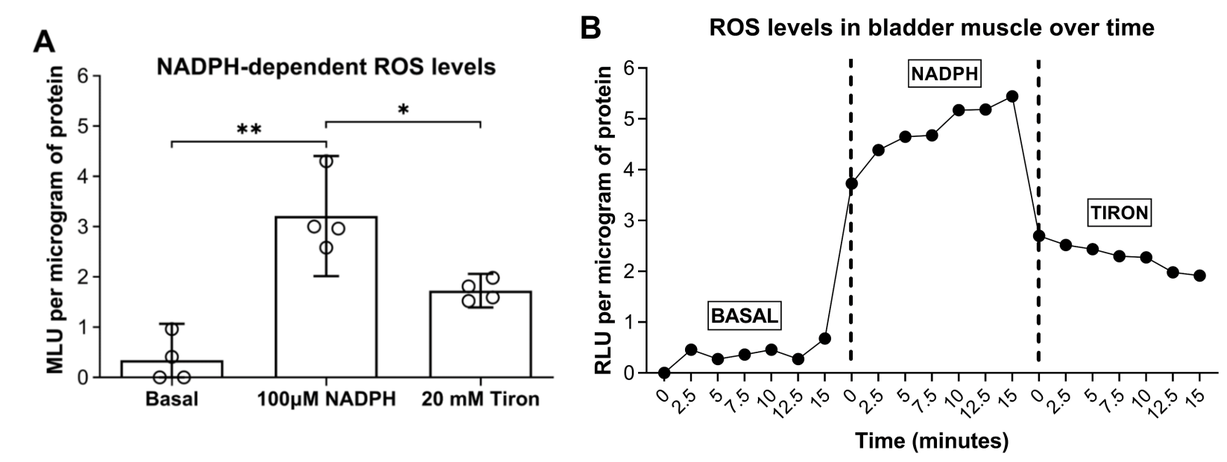

Figure 8. (A) Enhancement and attenuation of superoxide levels by NADPH, and Tiron, respectively. **(B)** Representative photon emission in response to the 3 different conditions (baseline (Basal), NADPH, and Tiron) measured over time (in minutes). MLU = mean light units. RLU = relative light units. *: $p < 0.05$, NADPH vs baseline or Tiron.

Discussion

- The enhancement of EFS-evoked contractions by exogenous ROS, hydrogen peroxide (H_2O_2) and the inhibition of these contractions by the Nox inhibitor and ROS scavenger, apocynin demonstrates the functional relevance of ROS in regulating human and dog bladder smooth muscle activity and suggests that endogenous Nox-derived ROS regulates smooth muscle function.
- The augmentation of contractions by the Nox activator and inflammatory mediator, angiotensin II (Ang II) suggests that activation of Nox via a receptor's ligand can also enhance smooth muscle activity and that the effect of Ang II is mediated by the 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.
- The excitatory effects of angiotensin II on bladder smooth muscle function may have significant pathological implications since inflammation is an important mechanism associated with oxidative damage.

References

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