Furosemide-induced dilation of pulmonary veins as a prophylactic for exercise-induced pulmonary hemorrhage

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ABSTRACT

Horses undergoing intense exercise are often diagnosed with exercised-induced pulmonary hemorrhage (EIPH). As a preventative, veterinarians routinely administer furosemide (Lasix™), prior to exercise. The use of Lasix™ is controversial since its mechanism of action is incompletely understood. Our research is aimed at elucidating pulmonary mechanisms by which furosemide is protective against EIPH. We hypothesized that furosemide induces dilation of pulmonary veins, through inhibition of the Na^+, K^+, Cl^- cotransporter, NKCC1. Pulmonary veins (2-4mm outer diameter) were isolated from the caudodorsal (CD) and cranioventral (CV) right lung lobe regions of nine horses. Each vessel was subjected to wire myography to assess dilation to furosemide (1e-6 to 1e-3 [logM]). As hypothesized, furosemide induced dilation of equine pulmonary veins taken from both Thoroughbreds (CD, 92 ±11%; CV, 83 ±10%) and other breeds (CD, 106 ± 12%; CV, 96 ±12% relaxation at 1e-3 [logM]). qPCR was used to determine mRNA expression of NKCC1. Veins isolated from both CV and CD portions of the lung had mRNA expression similar to positive control tissue (kidney, lung, spleen) in both Thoroughbreds and other breeds. In future, histology will be used to determine the protein location of the NKCC1 transporter in horse lung veins. In conclusion, preliminary findings indicate furosemide dilates isolated equine pulmonary veins *in vitro*, and that NKCC1 is expressed in pulmonary tissue. These data are the 1st ever to demonstrate furosemide has a direct effect on equine pulmonary vasculature, and that NKCC1 mRNA exists in the lung. These data suggest NKCC1 inhibition is a plausible pulmonary vein-mediated mechanism underlying the efficacy of furosemide for prophylaxis of EIPH in horses.

OBJECTIVE

Elucidate pulmonary mechanisms by which furosemide is protective against exercise-induced pulmonary hemorrhage.

HYPOTHESIS

Furosemide induces dilation of pulmonary veins, through inhibition of the sodium, potassium, chloride cotransporter, NKCC1.

METHODS

9 horses were euthanized → Collected CD and CV right lung lobe tissue → 2-4mm vein rings were isolated → Wire Myography / RNA isolation and PCR / Histology

	Horse #1	Horse #2	Horse #3	Horse #4	Horse #5	Horse #6	Horse #7	Horse #8	Horse #9
Breed	†Tennessee Walker	Hanoverian	TB	TB	TB	Quarter Horse	Quarter Horse	TB	TB
Age	1 year old	15 years old	4 years old	9 years old	5 years old	22 years old	22 years old	11 years old	22 years old
Sex	Gelding	Gelding	Mare	Mare	Gelding	Mare	Mare	Gelding	Gelding
Body Weight (lbs)	780	1290	958	1028	1102	1072	1084	1124	1086
Lung Weight (kgs)	-	-	5.1	5.55	5.05	4.7	*9.65	4.9	6.65

† chronic cystitis, bilateral ureteritis, bilateral hydronephrosis
* inflammation and accumulation of granulocytes due to history of heaves
TB=Thoroughbred

Table 1. Demographics of equine lung tissue donors

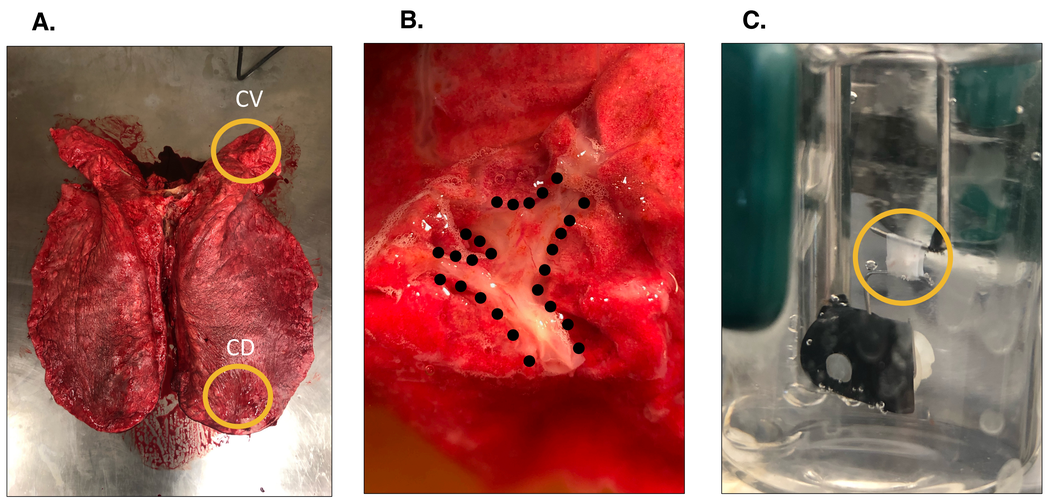


Figure 1: A) CD and CV equine lung tissue collection **B)** Pulmonary Vein Isolation **C)** Wire myography: Pulmonary vein is submersed in Krebs buffer and challenged with KCl while length-tension curves were generated, followed by U46619 (thromboxane A2 agonist) induced constriction and furosemide-induced dilation.

RESULTS

Table 2. Vessel size Thoroughbreds

Caudodorsal	Average	Standard Error	Cranioventral	Average	Standard Error
Length (mm)	4.46	0.18	Length (mm)	4.77	0.19
Inner diameter (mm)	1.88	0.20	Inner diameter. (mm)	1.74	0.16
Outer diameter (mm)	2.65	0.24	Outer diameter (mm)	2.69	0.17
Wall thickness (mm)	0.38	0.04	Wall thickness (mm)	0.47	0.06

Table 2. Vessel size other breeds

Caudodorsal	Average	Standard Error	Cranioventral	Average	Standard Error
Length (mm)	4.32	0.16	Length (mm)	4.44	0.18
Inner diameter (mm)	1.92	0.21	Inner diameter (mm)	2.12	0.22
Outer diameter (mm)	2.82	0.24	Outer diameter (mm)	3.06	0.26
Wall thickness (mm)	0.45	0.05	Wall thickness (mm)	0.47	0.06

Wire Myography

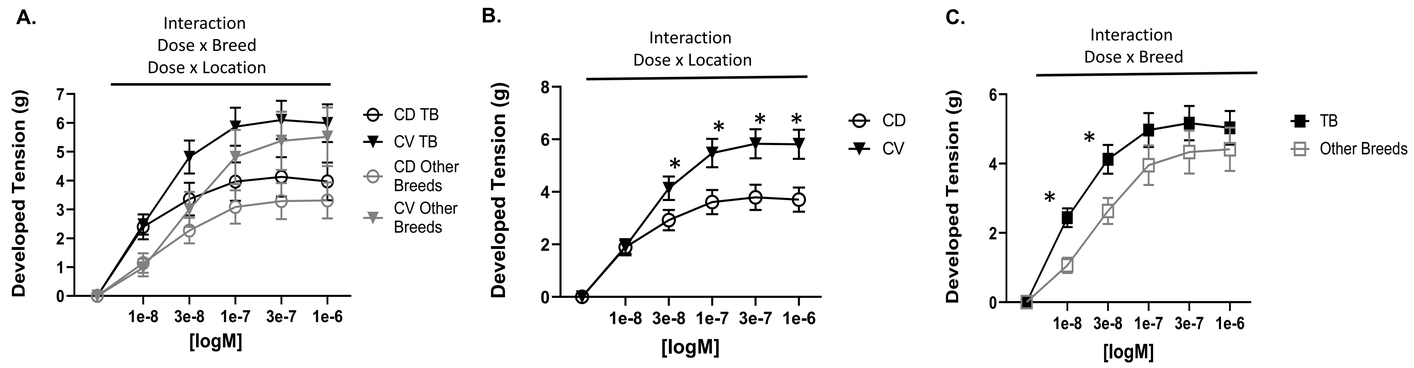


Figure 2. Thromboxane A2 Agonist (U46619) Induced Constriction is Dependent on both Breed and Location. (A) Developed tension in veins taken from the cranioventral (CV) and caudodorsal (CD) portions of the lung in Thoroughbreds (TB) and other breeds. (B) Developed tension in CV vs. CD (all breeds). (C) Developed tension in TB vs. other breeds (both locations). Developed tension was calculated using the following equation (CT-RT). CT= contractile tension. RT= resting tension. 3-way ANOVA (A), 2-way ANOVA (B,C), (*p<0.05).

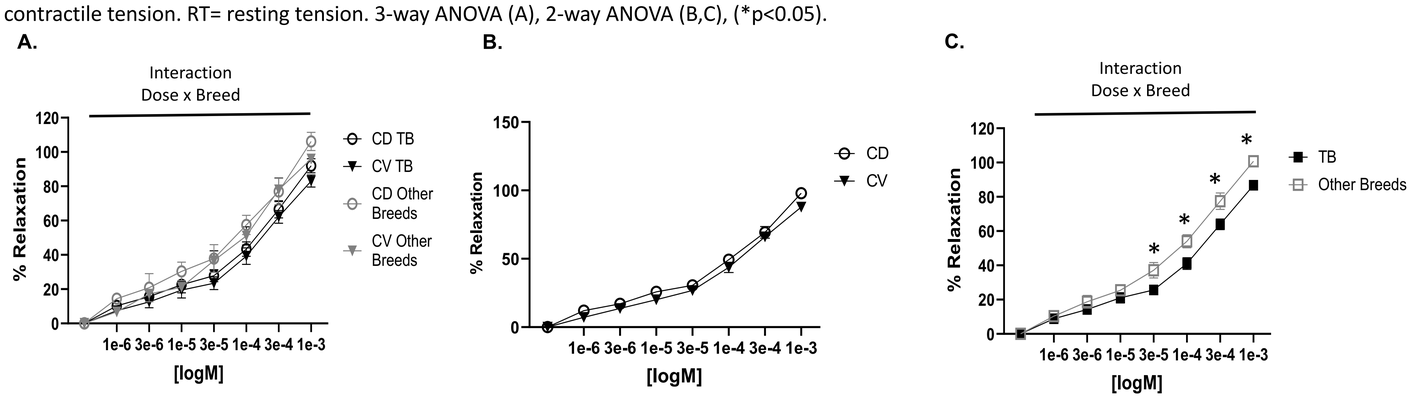
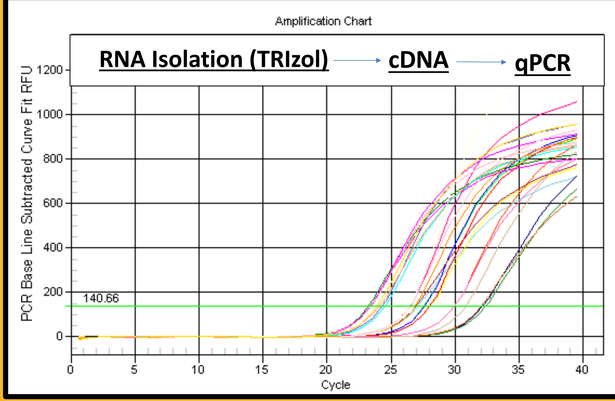


Figure 3. Furosemide Induced Dilation is Dependent on Breed but not Location. (A) % Relaxation in veins taken from CV and CD portions of the lung in TB and other breeds. (B) % Relaxation in CV vs. CD (all breeds). (C) % Relaxation in TB vs. other breeds (both locations). % Relaxation was calculated using the following equation ((CT-x)/(CT-RT))*100. x = measured grams of tension at each dose. 3-way ANOVA (A), 2-way ANOVA (B,C),(*p<0.05).



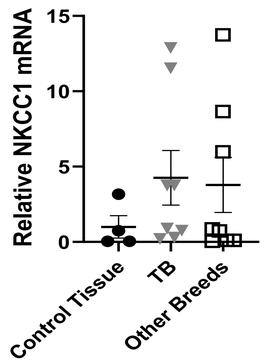


Figure 4. Relative NKCC1 mRNA expression in lung veins in TB and other breeds. Quantitative PCR (qPCR) was performed on a BioRad iCycler. NKCC1 and 18S primers were optimized for linearity and efficiency. The NKCC1 mRNA underwent multiple rounds of amplification and desired product was detected by fluorescence (A) and quantified by using the 2^-ddCT method (B) with the following equation: $2^{-(NKCC1\ CT - 18S\ CT)_{experimental\ group} - (NKCC1\ CT - 18S\ CT)_{control}}$.

CONCLUSION

These are the first data to demonstrate NKCC1 is expressed in equine pulmonary vasculature and that furosemide induces dilation of pulmonary veins. Therefore, NKCC1 inhibition is a plausible pulmonary vein-mediated mechanism underlying the efficacy of furosemide for prophylaxis of EIPH in horses.

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