Title

NADPH oxidase is the source of ROS in STZ rat aorta; use of the novel highly selective NOX inhibitor VAS2870

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Abstract text (max 400 words)

Abnormal vascular responsiveness in diabetes has been attributed to a number of changes in contractile and dilatory pathways, affected in part by the overproduction of reactive oxygen species (ROS). It has been reported that NADPH oxidase (NOX) is increased in endothelial cells of streptozotocin (STZ) treated rats, a model of diabetes. However pharmacological tools used in NOX studies are of limited use; the NOX inhibitor apocynin is non-selective and has off-target effects[1] and may inhibit nitric oxide synthase. Here we use the novel, highly selective NOX inhibitor VAS2870[2] to confirm the source of ROS in aorta of STZ rats.

Male Wistar rats (250 to 350g) were injected with 65mg/kg STZ; development of diabetes was confirmed after 5 days by testing blood glucose levels. Rats were killed by CO_2 asphyxiation, and the thoracic aorta removed and cleaned for mounting in a 20ml organ bath. Aorta were tensioned to 1g, equilibrated and incubated with 10^{-5}M VAS2870, 10^{-5}M apocynin, 150U/ml superoxide dismutase (SOD) or vehicle control (0.1% DMSO) in Kreb's buffer for 30 minutes, followed by an increasing concentration of phenylephrine (PE).

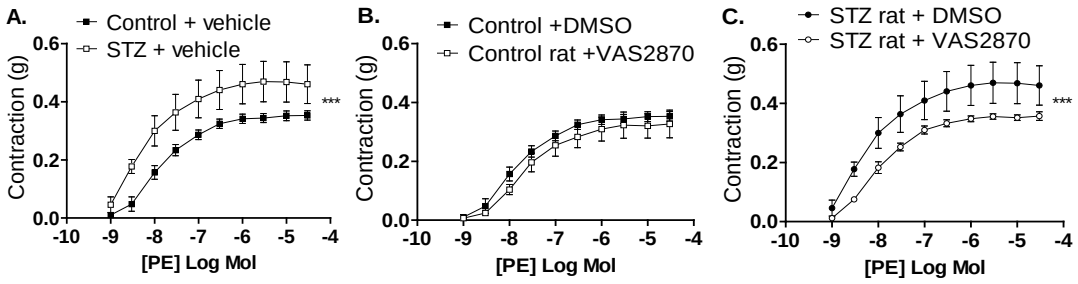

Figure 1. Response curve to PE in control and STZ treated rats (A); *aorta responses to PE following incubation with VAS2870 in aorta from control (B) and STZ treated (C) rats. ***p=<0.001 significance by two way ANOVA; n=4 for all groups*

PE induced contraction of STZ rat aorta is significantly increased in comparison to control rat aorta (Figure 1A). Incubation with VAS2870had no effect on PE induced contraction in control rat aorta (figure 1B), and significantly reduced contraction in STZ treated rat aorta to a level consistent with control rat aorta (figure 1C). In contrast, the apocynin had no significant effect on aorta from either type of rat. Contraction following incubation with SOD had no effect on control rat aorta (– SOD EC_{50} = $1.8x10^{-8}$M, E_{max}=0.353 ± 0.016g; +SOD EC_{50} = $2.5x10^{-8}$M, E_{max}= 0.338 ± 0.051g), and in contrast significantly reduced the contraction in STZ rat aorta (-SOD EC_{50} = $6.5x10^{-9}$M, E_{max}= 0.460 ±0.067; +SOD EC_{50} = $1.5x10^{-8}$M, E_{max}= 0.322 ± 0.037g).

VAS2870 reduces the PE overcontraction that is also reduced by SOD. This is consistent with the idea that the enhanced contraction is primarily due to ROS generated by NOX, and is the first report of the NOX inhibitor VAS2870 in STZ treated rats.

[1]Heumuller et al, Hypertension 2008, [2]ten Freyhaus et al, Cardiovasc Res 2006