Vasodilation and blood pressure-lowering effect mediated by 5,6-EEQ lactone in 5/6 nephrectomy hypertensive rats

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Introduction

- Vascular hypertension is a major, modifiable risk factor for all-cause mortality and morbidity worldwide.
- Microvascular dysfunction is a key contributor to vascular hypertension, leads to the loss of nitric oxide and impaired microvascular dilation.
- In these conditions endothelium-derived hyperpolarizing factors (EDHF's), constitute a compensatory dilation mechanism, regulate the microvascular tone.
- Among the EDHF's are the epoxyeicosatetraenoic acids (EEQs) region-isomers, epoxides derived from ω-3 polyunsaturated fatty acids.
- Previously, we demonstrated that 5,6 EET-lactone, arachidonic acid derivative, acts as an EDHF in human micro-vessels.

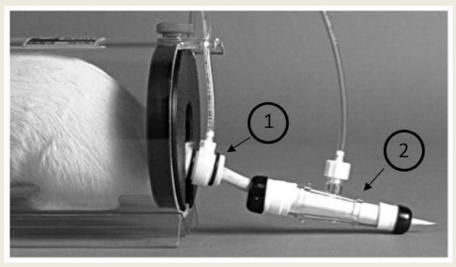
Hypothesis and aims

Based on structural similarities, we hypothesize that 5,6 EEQ –lactone derived from eicosapentaenoic fatty acid (EPA-L) may bare EDHF properties. Herein, we assessed EPA-L vasodilatory and BP-reducing impact, and its mechanism of action in hypertensive 5/6 nephrectomy (5/6Nx) rats.

Methods

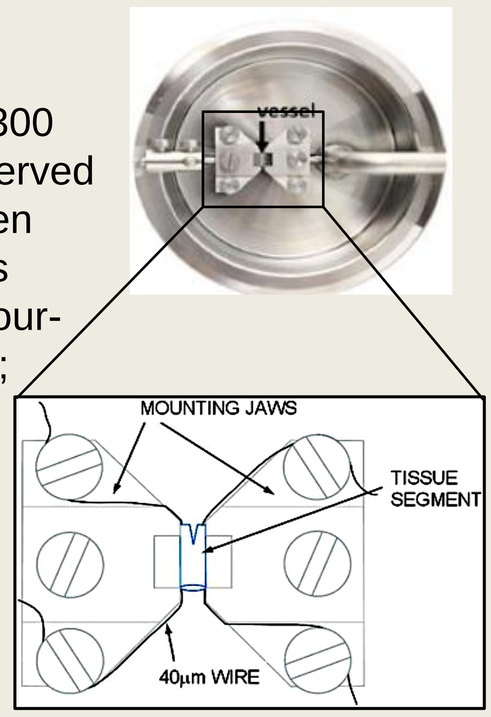
- *A*nimal experiments were conducted according to ethical committee guidelines (ethics #:26-04- 2017).
- *Hypertension inducted* using the 5/6Nx. Briefly, under deep anesthesia the vasculature of the right kidney was ligated and cut and so were two branches of the left renal artery, resulting ischemia of ~5/6 of body nephrons, i.e., 5/6Nx.

BP measurement:
Noninvasive BP measurements, performed in conscious rats using tail-cuff plethysmography (CODA non-invasive BP system, Kent Scientific Corporation, Torrington CT, USA).



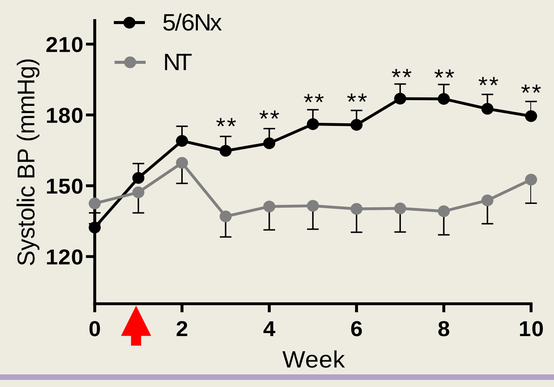
Kidney function:
24 hrs urine production collected in metabolic chambers (Techniplast S.p.A., Buguggiate, Italy). Water intake, food consumption and feces production and body weight (BW) were evaluated as well. Urine and blood chemical analysis produced full renal function profile, include creatinine clearance (CCr) and ion excretions (FE).

Microvascular reactivity:
Rats' intact second- and third-order branches of mesenteric arteries (250–300 μm) were carefully extracted, and preserved in physiological solution over night. Then each was placed on two tungsten wires (25-μm-diameter), and mounted on a four-chamber wire myograph (model 620 M; Danish Myo Technology, Hinnerup, Denmark). Endothelial function (force measurements) was assessed, by stimulation with various agents (KCl, thromboxane mimetic U- 46619, acetylcholine (Ach) and EPA-L), and relative relaxation response was calculated.



Results

5/6Nx induced significant systemic hypertension. Red arrow indicate surgery.
**P<0.01 *vs.* normotensive.



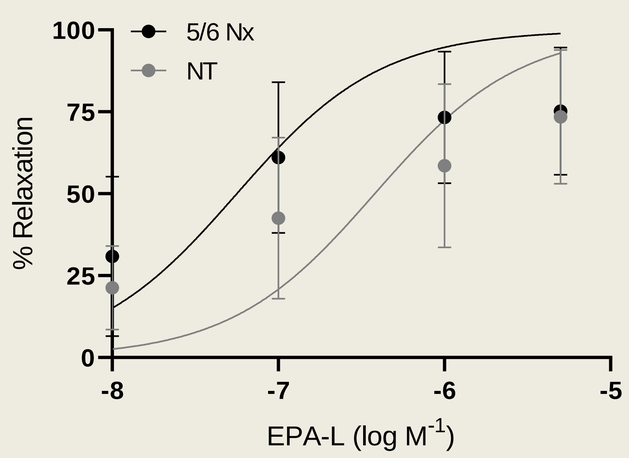
Physiological changes in 5/6Nx

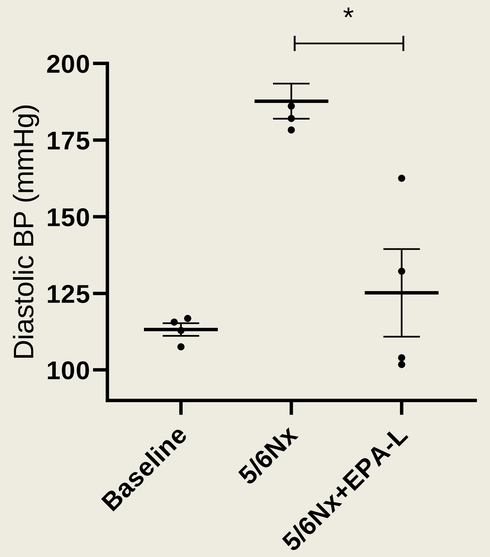
	Baseline (n=4)		5/6Nephrectomy (n=4)		5/6Nephrectomy + EPA-L (n=3)		
	Mean ± SD	Med	Mean ± SD	Med	Mean ± SD	Med	(†,*)p
BW (gr)	507 ± 28	520	450 ± 48 (††)	442	455 ± 33 (†)	448	<0.01
Glu (mg/dL)	141 ± 15	137	168±23(††)	163	194±41 (†††;**)	191	<0.01
BUN (mmol/L)	8 ± 1	8	20 ± 7(††)	18	26 ± 6(†††)	24	<0.01
U-pro (mg/dL)	553 ± 423	525	845 ± 244(††)	794	999 ± 364 (†††)	1172.60	<0.01
FEK (%)	29 ± 5	27	104 ± 44 (†††)	105	123 ± 63(†††)	133.69	<0.001

* compare to 5/6Nx; † compare to baseline using two-way ANOVA with Bonferroni posttest

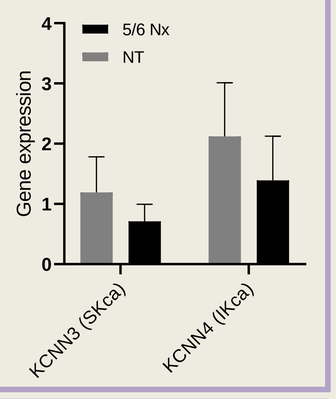
Arterial dilation and BP lowering by EPA-L

- EPA-L has a higher relaxation efficacy on arterioles taken from 5/6Nx hypertensive rats *vs.* normotensive control.
- EPA-L reduced significantly BP in 5/6Nx rats at chronic administration (5 consecutive days)





5/6Nx affected SK_{Ca} and IK_{Ca} channel gene expression. Quantitative analysis of relative mRNA of KCNN3 (SK_{Ca}) and KCNN4 (IK_{Ca}) in mesenteric arterioles P>0.05



Conclusions:

5/6Nx model induces sustained systemic hypertension. EPA-L reduces BP by improving micro-vessel dilation involving calcium-dependent potassium endothelial channels.