

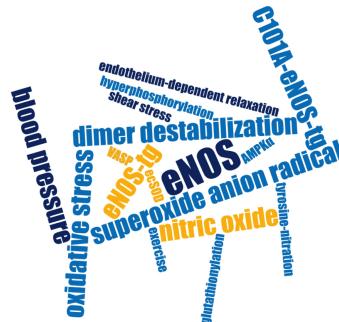


Stephanie Pick (Autor)

eNOS Derived Oxidative Stress and its Role in the Regulation of Blood Pressure

Stephanie Pick

eNOS Derived Oxidative Stress and its Role in the Regulation of Blood Pressure



Cuvillier Verlag Göttingen
Internationaler wissenschaftlicher Fachverlag

<https://cuvillier.de/de/shop/publications/7836>

Copyright:

Cuvillier Verlag, Inhaberin Annette Jentzsch-Cuvillier, Nonnenstieg 8, 37075 Göttingen,
Germany

Telefon: +49 (0)551 54724-0, E-Mail: info@cuvillier.de, Website: <https://cuvillier.de>



TABLE OF CONTENTS

Abbreviations	9
1. Introduction.....	13
1.1. Endogenous Nitric Oxide.....	13
1.2. Endothelial NO-Synthase	14
1.3. eNOS Signaling.....	18
1.4. Endothelium-Dependent Vasodilation	19
1.5. Oxidative Stress	20
1.6. eNOS and Blood Pressure	20
1.7. Aim of the Study	21
2. Materials and Methods.....	23
2.1. Substances and Solutions.....	23
2.2. Laboratory Animals.....	25
2.2.1. transgenic animals	26
2.2.2. genotyping of transgenic animals	27
2.2.3. physical exercise training	29
2.2.4. blood pressure and heart rate	30
2.2.5. treatment with Tempol®	31
2.2.6. preparation and organ removal	31
2.3. Functional Studies on Isolated Mouse Aorta	32
2.3.1. equipment and calibration.....	32
2.3.2. experimental protocol	34



TABLE OF CONTENTS

2.4.	eNOS mRNA-Expression.....	34
2.5.	Western Blot.....	37
2.5.1.	preparation of organs	37
2.5.2.	determination of protein concentrations.....	37
2.5.3.	general Western Blot protocol.....	38
2.5.4.	native gel electrophoresis.....	39
2.5.5.	antibodies.....	39
2.6.	Immunoprecipitation and Fluorescence Detection (Dynabeads®).....	41
2.7.	Detection of Superoxide Anion Radical	43
2.8.	Statistics	45
3.	Results	47
3.1.	Overexpression of eNOS	47
3.1.1.	genotyping.....	47
3.1.2.	eNOS protein expression	48
3.1.3.	eNOS dimer formation.....	49
3.1.4.	eNOS mRNA expression	50
3.2.	Oxidative Stress	51
3.2.1.	superoxide anion radical.....	51
3.2.2.	glutathionylated eNOS.....	53
3.2.3.	protein tyrosine-nitration	55
3.2.4.	phosphorylation of eNOS at Ser1176/9.....	57
3.3.	Functional Studies.....	62



TABLE OF CONTENTS

3.3.1.	endothelium-dependent relaxation	62
3.3.2.	endothelium-independent relaxation	63
3.3.3.	blood pressure and heart rate	64
3.3.4.	phosphorylation of VASP at Ser239	66
3.3.5.	ecSOD expression	68
3.4.	Effects of Tempol®	69
3.4.1.	eNOS protein expression	70
3.4.2.	detection of superoxide anion radical.....	71
3.4.3.	glutathionylated eNOS.....	72
3.4.4	eNOS Tyrosine-Nitration	73
3.4.5.	phosphorylation of eNOS at Ser1176/9.....	75
3.4.6.	blood pressure and heart rate	81
3.4.7.	phosphorylation of VASP at Ser239	85
3.4.8.	ecSOD expression	87
3.5.	Mechanical Studies	89
3.5.1.	exercise parameters	89
3.5.2.	eNOS protein expression	90
3.5.3.	gluthathionylated eNOS.....	92
3.5.4.	eNOS tyrosine-nitration	94
3.5.5.	phosphorylation of eNOS at Ser1176/9.....	96
3.5.6.	phosphorylation of VASP at Ser239	102



TABLE OF CONTENTS

4.	Discussion.....	105
4.1.	eNOS Mutation	106
4.2.	eNOS Protein Overexpression.....	107
4.3.	eNOS and Oxidative Stress.....	108
4.4.	eNOS Dimer-Destabilization and Aortic Reactivity	111
4.5.	eNOS and Blood Pressure	112
4.6.	eNOS and Physical Activity.....	113
4.7.	Posttranslational eNOS Modifications.....	114
4.8.	eNOS and the Role of Resistance Vessels.....	115
4.9.	Clinical Aspects	117
5.	Summary	119
5.1.	Abstract	119
5.2.	Abstract (deutsch)	121
6.	References.....	123
	Publications	133
	articles.....	133
	published abstracts / congress participations	135
	Curriculum Vitae.....	137
	Acknowledgements / Danksagung.....	138