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Original article

Sympathoadrenergic suppression improves heart function by upregulating the ratio of sRAGE/RAGE in hypertension with metabolic syndrome

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ABSTRACT

Receptors-for-Advanced-Glycation-End-products (RAGE) activate pro-inflammatory programs mediated by carboxymethyllysine (CML) and high-mobility-group-box1 protein (HMGB1). The soluble isoform sRAGE neutralizes RAGE-ligands preventing cardiovascular complications in conditions associated with increased sympathetic activation like hypertension and diabetes. The effects of sympathetic modulation on RAGE/sRAGE-balance and end-organ damage in metabolic syndrome on top of hypertension remains unknown. We hypothesized that increased sympathoadrenergic activity might lead to an unfavourable RAGE/sRAGE regulation. Renal denervation (RDN) was used to modulate sympathetic activation in obese spontaneously hypertensive rats (SHRobRDN) versus sham-operated obese spontaneously hypertensive rats (SHRob), their hypertensive lean controls (SHR) and non-hypertensive controls. Cardiac fibrosis was assessed by histological analysis and sRAGE/RAGE and ligand levels by Western blotting. Levels of CML and HMGB1 were highest in SHRob and were significantly lowered by RDN in serum (−44% and −45%) and myocardium (−25% and −52%). Myocardial RAGE was increased in SHR (+72% versus controls) and in SHRob (+68% versus SHR) while sRAGE decreased (−50% in SHR versus controls and −51% in SHRob versus SHR). RDN reduced myocardial RAGE expression. (−20%) and increased sRAGE levels in heart (+80%) and serum (+180%) versus sham-operated SHRob. Myocardial fibrosis correlated inversely with myocardial sRAGE content ($r = -0.79$; $p = .004$; $n = 10$). Myocardial sRAGE shedding active A-Disintegrin-And-Metalloprotease-10 (ADAM-10) was decreased in SHR (−33% versus controls) and in SHRob (−54% versus SHR), and was restored after RDN (+129% versus SHRob). Serum ADAM-10 activity was also decreased in SHRob (−66% versus SHR) and restored after RDN (+150% versus SHRob). In vitro, isoproterenol induced a ß1-adrenergic receptor mediated increase of RAGE expression in splenocytes (+200%) and decreased sRAGE secretion of splenocytes and cardiac fibroblasts (−50% and −49%) by ß2-adrenergic receptor stimulation mediated suppression of ADAM-10 activity. In conclusion, sympathetic activity affects sRAGE/RAGE-balance, which can be suppressed through sympathetic modulation by RDN, preventing RAGE-induced cardiac damage in hypertension with metabolic syndrome.

1. Introduction

The multi-ligand receptor RAGE (Receptor-for-Advanced-Glycation-End-products) was initially described as the signal-transducing receptor for advanced glycation end products (AGEs) and is involved in microvascular complications and end-organ damage associated with increased sympathetic activation in hypertension, diabetes and heart failure [1–6]. The soluble form of RAGE (sRAGE) comprises only the RAGE ectodomain and acts as a ligand decoy, competitively inhibiting RAGE activation and oxidative stress [1, 6]. Endogenous soluble RAGE isoforms are circulating in plasma and tissues and are suspected to inversely reflect cellular RAGE activity [7], with most sRAGE being produced by ADAM-10 mediated RAGE cleavage [8–9]. Whether modulation of sympathetic activation is responsible for the observed changes in RAGE/sRAGE balance, however, is unknown. Renal sympathetic denervation (RDN) reduces afferent and efferent sympathetic

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Table 1
Metabolic parameters, left ventricular and renal function.

	Control	SHR	SHRob	SHRobRDN	Control Vs. SHR	SHR Vs. SHRob	SHRob Vs. SHRobRDN	Control Vs. SHRob RDN
	n = 5–6	n = 6–7	n = 5–6	n = 5–6				
Parameters					P-value			
Creatinine umol/l	21 ± 1.3	29 ± 0.9	32.3 ± 5.3	21.3 ± 7.3	0.062	0.437	0.038	0.958
GFR l/kg/h	0.6 ± 0.02	0.4 ± 0.02	0.2 ± 0.02	0.3 ± 0.07	< 0.001	< 0.001	0.019	< 0.001
EF (%)	66.0 ± 1.8	51.2 ± 1.2	45.7 ± 1.6	54.1 ± 1.2	< 0.001	0.041	0.007	< 0.001
LVedP mm Hg	4.7 ± 1.4	6.0 ± 2.7	16.1 ± 2.3	7.4 ± 2.2	0.703	0.012	0.051	0.494
RR (MAP) mm Hg	116 ± 2.9	199 ± 5.6	224 ± 5	179 ± 12.2	< 0.001	0.031	< 0.001	< 0.001
HR bpm	328 ± 1.3	305 ± 13.7	276 ± 9.6	277 ± 13.4	0.285	0.119	0.994	0.026
HW g	1.55 ± 0.05	1.64 ± 0.07	1.61 ± 0.06	1.66 ± 0.07	0.234	0.738	0.646	0.214
BW g	618 ± 8.4	419 ± 11	697 ± 20.2	632 ± 36.7	< 0.001	< 0.001	0.157	0.999
HW/100 g BW ratio	0.24 ± 0.004	0.39 ± 0.011	0.23 ± 0.016	0.27 ± 0.025	< 0.001	< 0.001	0.094	0.201
Insulin Pg/ml	854 ± 231	1157 ± 294	10,126 ± 1518	7714 ± 1289.3	0.809	< 0.001	0.118	< 0.001
Glucose mmol/l	5.0 ± 0.11	5.76 ± 0.17	5.99 ± 0.17	5.13 ± 0.37	0.055	0.571	0.038	0.569
HbA1C % (mmol/mol)	3.9% ± 0.01 (19 ± 0.05)	3.6% ± 0.04 (16 ± 0.18)	3.7% ± 0.1 (17 ± 0.46)	3.7% ± 0.14 (17 ± 0.64)	0.132	0.464	0.462	0.879
Renal Norepinephrine pg/mg	103.5 ± 14.7	98.2 ± 2.3	108.7 ± 8.9	10.9 ± 2.4	0.628	0.634	< 0.001	< 0.001

GFR: glomerular filtration rate.
EF: left-ventricular ejection fraction.
LVedP: left-ventricular end-diastolic pressure.
MAP: mean arterial pressure.
HR: heart rate.
HW: heart weight.
BW: body weight.

nerve activity and has been developed to lower blood pressure in patients with uncontrolled hypertension [10–12]. Experimental and observational data suggest additional effects beyond blood pressure lowering such as reduction in myocardial hypertrophy, improved glucose tolerance and amelioration of microalbuminuria [13–16]. We have recently characterized a rat model of metabolic syndrome with hypertension, the spontaneously hypertensive obese rat (SHRob) carrying an additional mutation in the leptin receptor [17, 18]. In addition to hypertension, SHRob express further abnormal phenotypes including obesity, hyperinsulinemia and hyperlipidemia, a moderate decrease in LV-systolic function and diastolic dysfunction [18]. The exact mechanisms of how metabolic syndrome on top of hypertension results in more pronounced end-organ damage are still unknown. However, sympathetic modulation by RDN in SHRob for example has led to an improvement in renal and cardiac function [19].

The present study aimed to investigate the effect of in-vivo modulation of sympathoadrenergic activity by RDN on the cardiac ligand-RAGE/sRAGE-axis and subsequent cardiac remodelling in a rat model of metabolic syndrome on top of hypertension. In order to control for blood-pressure confounding, β-adrenergic receptor mediated RAGE/sRAGE regulation was studied in isolated rat mononuclear cells (splenocytes) and cardiac fibroblasts.

2. Materials and methods

2.1. Animals

Male obese spontaneously hypertensive rats (SHRob), their heterozygous hypertensive control rats (lean SHR) and non-hypertensive non-obese control rats (Sprague Dawley; controls) were purchased from Charles River GmbH (Sulzfeld, Germany) at the age of 10 weeks. The animals were housed individually in standard cages and received standard chow (standard diet #1320, Altromin, Lage, Germany) and tap water ad libitum. RDN or sham-operation was performed in SHRob at the age of 32 weeks and rats were killed at the age of 45 weeks. For the isolation of primary rat cardiac fibroblasts, Sprague Dawley rats from Charles River GmbH (Sulzfeld, Germany) aged 52 weeks were sacrificed instantly under general deep anaesthesia. All animal studies were performed in accordance to the German law for the protection of animals. Furthermore, the investigation conforms with the guide for the Care and Use of laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The study was approved by the regional commission in charge (Darmstadt, Germany).

2.2. Reagents

See online supplement.

2.3. Renal denervation

Renal denervation (RDN) was performed in SHRob at the age of 32 weeks (SHRobRDN) when metabolic syndrome was fully established to test the role of renal sympathetic innervation on the further progression of cardiac end-organ damage. The detailed procedure is described in the online supplements.

2.4. Metabolic cages, cardiac MRI and invasive functional measurements, sample asservation, histological analysis and immunoblotting

The protocols are described in detail in the online-only data supplement.

2.5. Isolation of primary rat cardiac fibroblasts and splenocytes, culture conditions, cell fractionation, protein and gene expression analyses

Detailed protocols are provided in the online supplements.

2.6. Statistics

Statistical analysis was performed with Graph Pad Prism (version 5.0; GraphPad Software, San Diego California, USA). Results are presented as means ± SEM. Significance was estimated with two-way-ANOVA with Fisher-LSD post hoc test for multiple comparisons. Normal contribution of data was tested by Kolmogorov-Smirnov and Lilliefors test and Spearman's or Pearson's correlations are shown respectively. A value of $p < .05$ was considered significant.