Angiotensin II-Derived Hydrogen Peroxide Impairs Endothelial Function in a Murine Model

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Excessive production of reactive oxygen species (ROS) leads to inactivation of nitric oxide (NO) and subsequent endothelial dysfunction. All cardiovascular risk factors (hypertension, dyslipidemia, diabetes, etc.) have been reported to elicit vascular oxidative stress. Angiotensin II (Ang II) is one of the most important mediators of vascular dysfunction in the presence of oxidative stress. Most of the actions of Ang II in endothelial cells are known to be associated with increased production of superoxide anion (O2–) which is the major responsible for endothelial NO synthase uncoupling. The aim of the present study was to assess the contribution of hydrogen peroxide (H2O2) production in murine vessels to endothelial dysfunction after Ang II treatment. Rat aortic segments were incubated in the presence vs. absence of Ang II or H2O2. Subsequently, the tissue was either studied in organ bath system for the vasomotor function or used for measurements of H2O2 production by the FOX (Ferric iron Xylenol Orange) assay. The results showed an attenuation of vascular relaxation response after Ang II application; this effect was at least partially mediated via the increased H2O2 generation and involved the impairment of NO production.

Keywords: experimental endothelial dysfunction, angiotensin II, hydrogen peroxide

Several vasomotor mediators are released by the endothelial layer: i) vasodilators – prostaglandins, nitric oxide (NO) and endothelin-derived-hyperpolarizing factor, and ii) vasoconstrictors – tromboxane and endothelin [1]. NO plays a key role in vascular homeostasis via the control of vascular tone, inhibition of platelet aggregation, regulation of local cell growth, thus maintaining the physiological endothelial function [2, 3].

The vast majority of cardiovascular risk factors (hypertension, dyslipidemia, diabetes, etc.) have been reported to elicit vascular oxidative stress. Angiotensin II (Ang II) is one of the most important mediators of vascular dysfunction in the presence of oxidative stress. Most of the actions of Ang II in endothelial cells are known to be associated with increased production of superoxide anion (O2–) which is the major responsible for endothelial NO synthase uncoupling. The aim of the present study was to assess the contribution of hydrogen peroxide (H2O2) production in murine vessels to endothelial dysfunction after Ang II treatment. Rat aortic segments were incubated in the presence vs. absence of Ang II or H2O2. Subsequently, the tissue was either studied in organ bath system for the vasomotor function or used for measurements of H2O2 production by the FOX (Ferric iron Xylenol Orange) assay. The results showed an attenuation of vascular relaxation response after Ang II application; this effect was at least partially mediated via the increased H2O2 generation and involved the impairment of NO production.

The aim of the present study was to assess the effect of Ang II treatment on H2O2 production in isolated murine vessels.

Experimental part

Materials and methods

Study design and animal procedures

Experiments were performed in vascular preparations (thoracic aortas) isolated from male Sprague-Dawley rats weighing 250-350 g (n=6/group). The arterial segments were carefully cleared of connective tissue so that to damage not the intimal surface and cut into 2-3 mm wide rings.

Animals were fed ad libitum and housed under standard conditions (constant temperature and humidity of 22.5 ± 2°C and 55 ± 5%, 12-h light/dark cycle). Twenty-four hours prior the experiment solid food was withdrawn with no limitation in water supply.

All experimental procedures used in this study were conducted in accordance with the Directive 2010/63/EU on the protection of animals used for scientific purposes. The experimental protocol was approved by the Committee for Ethics Research of “Victor Babes” University for Medicine and Pharmacy of Timișoara, Romania.

Most reagents were from Sigma Aldrich, Germany.

Organ Culture

Rat aortic segments were dissected under sterile conditions, cleaned, and incubated for the times indicated at 37°C in EBM culture medium containing 0.1% BSA, in the presence or absence of Ang II (100 nmol/L) or hydrogen peroxide (100 microm).
Organ Bath Experiments

Organ bath experiments were performed in aortic rings in the presence of diclofenac (10 μmol/L). The concentration of phenylephrine (Phe) used for preconstriction was adjusted to obtain an identical preconstriction level of 80% of the contraction elicited by KCl (80 mmol/L). Endothelium-dependent relaxation to increasing concentrations of acetylcholine (ACh) was registered. It was also tested the vascular contractility to L-NAME (Nω-Nitro-L-arginine methyl ester hydrochloride, 10 μM).

Reactive Oxygen Species Measurement

Hydrogen peroxide production was measured in rat aortic segments by means of FOX (Ferric iron Xylenol Orange) oxidation method using PeroxyDetect Kit (Sigma Aldrich). The principle of the assay is that peroxides oxidize Fe2+ to Fe3+ ions at acidic pH. The Fe3+ ion will form a colored adduct with xylenol orange (XO, 3,3’-bis[N,N-bis(carboxymethyl)aminomethyl]-o-cresolsulfonphthalein, sodium salt), which is observed at 560 nm.

Statistics

All values are mean±SEM. Relaxations were calculated from individual dose-response curves. Statistical analysis was carried out by ANOVA or ANOVA for repeated measurements followed by Fisher LSD posthoc test. Values of p<0.05 were considered statistically significant.

Results and discussions

In vitro Exposure to Ang II Results in Vascular Function Impairment

In rat aortic segments incubated for 24 h with Ang II we measured the vascular contractility and relaxation responses in the organ bath setup (Schuler organ bath). The vascular contractility after Ang II stimulation was significantly increased in response to cumulative doses of phenylephrine (p<0.05 vs. control, fig.1A). Also, endothelium-dependent relaxation, after Ang II pretreatment was significantly attenuated (p<0.05 vs. control, fig.1B). In Ang II-treated segment the contractility to L-NAME (Nω-Nitro-L-arginine methyl ester hydrochloride, 10μM) was significantly increased (fig.1C), observation suggestive for the important role of NO in vascular vasomotor function. Indeed, by inhibiting eNOS, less NO production and vascular relaxation [13, 14]. However, since hydrogen peroxide increased the endothelial level of calcium with subsequent activation of eNOS, NO production and vascular relaxation [13, 14]. However, since hydrogen peroxide is freely diffusible through cell membranes, in high concentrations it can further activate inflammation and promote endothelial dysfunction [15].

Pretreatment with High Concentration of H2O2 Leads to Vascular Dysfunction

The most investigated radical species in relation with endothelial dysfunction is O2.− produced by several enzymatic pathways. In this study we aimed to investigate the effect of H2O2 on the vasomotor function. In this regard we incubated overnight rat aortic segments with H2O2, and evaluated the endothelium-dependent relaxation in organ bath. We obtained a significant reduction of the vasodilator response, suggestive for the deleterious effects of H2O2 in high concentration (fig. 2).

Exposure to Ang II increase H2O2 production

Since exogenous H2O2 administration elicited endothelial dysfunction, we further investigated whether treatment with Ang II could be responsible for the increased endogenous production of H2O2. The results of FOX assay showed a significant increase in H2O2 production after Ang II stimulation.

In the present study we assessed the vasomotor function and H2O2 production of rat aortic segments after Ang II stimulation. We reported an attenuation of relaxation in response to Ang II pretreatment, an effect that was, at least partially, mediated by the increased H2O2 generation that in turn induced an impairment of NO production. This effect is supported by an excessive augmentation of contractility to L-NAME as demonstrated by the study on vasomotor function.

It is widely accepted that the main free radical with unequivocal role in the progression of endothelial dysfunction is the superoxide anion (O2−). Indeed, O2− is considered the only responsible for scavenging NO with the generation of peroxynitrite that further triggers eNOS uncoupling [11, 12].

In physiological concentrations H2O2 has been reported to exert beneficial effects on vascular function. In particular, hydrogen peroxide increased the endothelial level of calcium with subsequent activation of eNOS, NO production and vascular relaxation [13, 14]. However, since hydrogen peroxide is freely diffusible through cell membranes, in high concentrations it can further activate inflammation and promote endothelial dysfunction [15].

Treatment with Ang II is a classic method to induce experimental endothelial dysfunction [16] via activating several enzymatic systems that generate either O2 or H2O2.

was calculated as the difference between the maximum contractility to L-NAME and the value that represents 10% from the maximal KCI contraction in response to Phe.

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The major ROS sources are NADPH oxidases [17] and mitochondrial respiratory chain [18]. Superoxide anion is produced by: isofrom 2 of NADPH (nicotinamide-adenine dinucleotide phosphate) oxidase [19, 20], uncoupled endothelial NO synthase (eNOS), cyclooxygenase, defective mitochondrial electron transport [21], whereas isofrom 4 of NADPH oxidase [19, 20], xanthine oxidase [22] and, more recently, p66 [23] and monoamine oxidases [24] are responsible for hydrogen peroxide generation. All these enzymatic systems contribute to the aggravation of endothelial dysfunction and the progression of atherosclerotic vascular disease.

Despite the fact that we confirmed the contribution of AngII-derived H2O2 to the development of the in vitro endothelial dysfunction, no data are provided about the intracellular sources of H2O2, which is a limit of our study.

Conclusions

The present study performed in murine vascular rings exposed to angiotensin II demonstrated that increased generation of hydrogen peroxide elicited an impairment of the nitric oxide-dependent relaxation. Whether this observation can be recapitulated in vascular rings harvested from diabetic vs. non-diabetic patients with endothelial dysfunction as well as the signal transduction pathways is a topic of our current research.

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References


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