Apocynin activity in spontaneously hypertensive rats (SHR): preliminary studies in vivo

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Abstract An elevation in angiotensin II (Ang II) levels is a common occurrence in spontaneously hypertensive rats (SHRs). Infusions of Ang II and a high salt diet increase the activity of NADPH oxidase that stimulates superoxide anion (O^{2-}) generation and increases the expression of certain subunits of NADPH oxidase. Apocynin, an NADPH oxidase inhibitor with antihypertensive effects, is able to inhibit the release of superoxide anion by inhibiting NADPH oxidase activity and blocking the migration of p47 phox to the mitochondrial membrane. The aim of our study was to evaluate the antihypertensive effects of apocynin in SHRs and Wistar rats (WKYs) using a micropuncture technique. After microperfusion of both the proximal and distal tubules, we found that SHRs treated with apocynin showed a decrease in the free-flow collection of the proximal tubule (PT), which was not affected in WKYs. Moreover, significant differences were not demonstrated in the distal tubule (DT), probably due a mechanism of compensation that occurs in the loop of Henle. In conclusion, it is possible that the mechanisms of reabsorption in the PT are controlled by the interactions of O^{2-} and nitric oxide (NO). These data could suggest a higher activity of NADPH oxidase and increase in reactive oxygen species (ROS) production in the PT during hypertension.

Keywords Apocynin · Hypertension · Microperfusion · Kidney

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Abbreviations

SHR	spontaneously hypertensive rat
WKY	Wistar-Kyoto rats
ANG	II angiotensin II
ROS	reactive oxygen species
O ⁻²	superoxide anion
PT	proximal tube
DT	distal tube
TGF	tubuloglomerular feedback
bw	Body weight

Introduction

SHRs are spontaneously hypertensive rats in which an elevation of angiotensin II (ANG II) level is a common occurrence. Harrison et al. (2003) have shown that the hypertension induced by ANG II correlates with oxidative stress, implying an increase of reactive oxygen species (ROS). Infusions of ANG II (Wang et al. 2003) and high salt diets (Kitiyakara et al. 2003) increase the activity of NADPH oxidase that stimulates superoxide anion (O⁻²) generation and also improved the expression of some subunits of NADPH oxidase (p22 phox and p47 phox proteins). Furthermore, the involvement of NADPH oxidase during hypertension was observed, where the p47 phox protein appears to be an important factor for the generation of ROS (Babior 1999). NADPH oxidase is widely expressed in the kidney, and is present in blood vessels, interstitial cells, glomerulus, and tubules. The pathophysiological importance of this enzyme has led many researchers to use some NADPH oxidase inhibitors, in particular apocynin, also known as acetovanillone [4-hydroxy-3-methoxyacetophenone], a natural organic compound with multiple pharmacological properties. Apocynin has antihypertensive effects. It is able to inhibit release of superoxide anions by inhibition of NADPH oxidase activity and blocks migration of p47 phox to the mitochondrial membrane (Babior 2004). O⁻² generated by NADPH oxidase could be used for the production of ROS used by phagocytes or O⁻² could be used as a second messenger in non-phagocytic cells (Li et al. 2005). Due to the selectivity of its inhibition, apocynin could be widely used as an inhibitor of NADPH oxidase without interfering with other aspects of the immune system. The aim of this study was to evaluate *in vivo* the antihypertensive effects of apocynin in SHR and Wistar-Kyoto rats (WKY) rats using a micro-perfusion technique.

Materials and methods

Sixteen male SHR and 16 male WKY rats weighting 150±15 g were housed under constant environmental conditions of 22°C with a 12-h light–dark cycle. Rats were subdivided into four groups, each consisting of 8 animals, and treated for 24 h as follows: Group I – SHR control; Group II – WKY control; Group III – SHR treated with apocynin orally (16 mg/Kg/die); Group IV – WKY treated with apocynin orally (16 mg/Kg/die). The rats were anesthetized intraperitoneally with thiobarbital (Inactin; Sigma Aldrich, St. Louis, MO) (120 mg/kg/bw) and prepared for the micro-puncture study of the left kidney using a thermo-regulated table (37°C). For the tracheotomies, the jugular veins were catheterized with polyethylene PE-50 tubing that was used for intravenous infusion of saline solution

Fig. 1 Blood pressure values in SHR and in WKY before and later the treatment. Data represents mean $\pm$ S.D. of 8 rats. * $P < 0.0015$ vs. control. SHR have a significant increase of blood pressure correlated with WKY

with an infusion pump (Braun, Melsungen, Germany). The femoral artery was catheterized to record the mean blood pressure with a pressure transducer (Powerlab, AD Instruments Inc, Colorado Springs, CO, USA). The bladder and ureter were catheterized with PE 90 and PE 10 polyethylene tubes, respectively, for collecting urine to evaluate kidney functionality. The left kidney was exposed by a flank incision and stabilized in a Lucite cup. After 45 min of equilibration, a micropipette containing mineral oil was inserted between the first and third loop of the proximal tubule. A drop of this oil was injected into the proximal tubule to follow the course of the flow and to make the proximal tubule block. The free flow collections of the medium proximal tubule and distal tubule were performed for a period of 3–4 min. The samples were then transferred to the capillaries and measured by calibrated microscope.

Results

Blood pressure and urinary flow

Systolic blood pressure was 139 ± 0.57 mm Hg in SHR and 112 ± 11.2 mm Hg in WKY rats ($p < 0.0015$). Apocynin treatment did not induce any significant changes. Urine flows were similar in all groups.

Free-flow collections of proximal tubule (PT) and distal tubule (DT)

PT flow was similar in SHR as compared to normotensive rats (14.01 ± 2.7 nl/min in SHR, 12.01 ± 3.6 nl/min in WKY). However, PT flow was substantially reduced in SHR treated with apocynin (6.97 ± 1.7 nl/min) as compared to WKY treated with apocynin (14.59 ± 3.2 nl/min). Additionally, there were not significant differences of DT flow in SHR and

Fig. 2 Free flow collections of the proximal (left panel) and distal tube (right panel). Data represent mean $\pm$ S.D

WKY before or after treatment ($\text{SHR} 5.32 \pm 1.2$ nl/min, WKY 7.05 ± 0.8 nl/min, SHR with Apo 5.08 ± 0.8 nl/min, and WKY with Apocynin 5.92 ± 0.3 nl/min).

This preliminary study shows that SHR rats treated with apocynin had decreased free flow in the proximal tubule, while significant difference was possible to see in WKY rats. In the distal tubule there were no differences in flow between all groups. This reduction in the proximal tubule was potentially compensated due to tubuloglomerular feedback (TGF) activation (Welch and Wilcox 1992).

Discussion

One new finding in this preliminary study was that PT flow is similar in SHR and WKY rats, which correlates with previous studies (Arendshorst and Beierwaltes 1979). The failure to detect basal differences in PT flow during hypertension remains unclear. This could be due to the method used in this study which blocks flow to the macula densa and tends to increase the glomerular filtration rate (GFR) and therefore, PT flow in both strains. Direct measurements of absolute fluid reabsorption by in situ microperfusion is the most accurate method to characterize PT function, since this method separates transport from the confounding effects of PT flow and TGF. Apocynin directly blocked NADPH oxidase, the most important source of O^{-2} . Treatment with this drug significantly reduced the PT flow in SHR.

The aim of this work was to investigate the involvement of the NADPH oxidase during hypertension. Our data show that acute treatment with apocynin significantly reduced the proximal tubule flow. It is possible that the mechanisms of reabsorption in PTs are controlled by the interactions of O^{-2} and nitric oxide (NO). Renal levels of O^{-2} and NADPH oxidase are higher in SHR as compared with WKY rats (Welch et al. 2001). In conclusion, these data suggest an increased activity of NADPH oxidase and increased ROS production in the proximal tubule during hypertension (Figs. 1 and 2).

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