The role of AT1 and AT2 angiotensin receptors in the mechanism of apoptosis in renal tubular cells after physical exercise

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Abstract

Intensive physical exercise disturbs the entire homeostasis in the body and leads to changes in haemodynamic and metabolic alterations not only in skeletal muscles but also in many distant organs. In response to acute physical exercise, a decrease of the glomerular filtration may occur, followed by stimulation of the renin-angiotensin system (RAS). Recent studies have shown that both AT1 and AT2 angiotensin receptors may play a role in mediating the apoptotic process in the kidney. Our previous studies have demonstrated an occurrence of apoptosis in rat renal tubular cells after an excessive exercise. The aim of the present study was to determine the possible mechanism of exercise-induced apoptosis in rat kidney. The analysis was performed on kidneys of rats, subjected to treadmill running until exhaustion. Apoptosis was detected in paraffin sections by the TUNEL technique. The expression of AT1 and AT2 receptors in renal tubular cells was examined by immunohistochemistry and Western blot. Our results confirmed that apoptosis after physical exercise is present in renal distal tubular cells. Moreover, there was an increased expression of AT1 and AT2 receptors in distal tubular cells. These studies suggest that physical exercise may induce apoptosis by a mechanism, involving the activation of angiotensin AT1 and AT2 receptors.

Key words: angiotensin receptors, apoptosis, kidney, physical exercise.

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Introduction

Intensive physical exercise disturbs the entire homeostasis in the body and leads to changes in haemodynamic and metabolic alterations not only in skeletal muscles but also in many distant organs, such as the kidney or the heart. A decrease of glomerular filtration, sodium depletion and increased blood concentrations of catecholamines (adrenaline, noradrenaline) are among the effects of excessive physical exercise. All of these mechanisms could activate the juxtaglomerular apparatus to renin secretion. Moreover, there are many tissues, including vasculature's endothelial and smooth muscle cells, the heart and the kidney, which have their own local renin-angiotensin (RAS) systems, capable of producing angiotensin II in response to metabolic changes, occurring after physical exercise. Elevated blood renin levels (plasma rennin activity, PRA) stimulate the conversion of angiotensinogen to angiotensin I, which is then converted to active angiotensin II by the angiotensin-converting enzyme within the pulmonary circulation. Angiotensin II acts through its receptor subtypes, type 1 (AT1) and type 2 (AT2) receptors, which involve different molecular mechanisms. Despite the place of secretion, angiotensin II exerts a wide range of actions, including apoptosis promotion. A stimulation of AT1 and AT2 receptors may result in either an increased expression of p53 protein (associated with the activation of the transcriptional nuclear factor kappa B - NF-κB) or in a generation of reactive oxygen species (ROS). Both of them could promote the apoptotic process [1, 2]. Our previous study demonstrated the presence of apoptotic-damaged cells in the kidney after physical exercise [3]. In the present study, we examined the possible role of AT1 and AT2 receptors in the induction of apoptosis of renal tubular cells after an intensive physical exercise.

Material and Methods

Eighteen male Wistar rats, 10-12 weeks of age (200-250 g body weight), formed groups of running (N=12) and not running

Table 1. Apoptosis in kidney tubular cells. Control: notrunning animals. 6h: running animals killed after 6h. 96h: running animals killed after 96h. Significant differences: the control values, as compared to respective values after 6h *p<0.01; the control values, as compared to respective values after 96h **p<0.001.

Apoptotic cell nuclei in 500 examined tubular cells	Control	6h	96h
(%)	1.32 ± 0.3	4.69 ± 0.7 *	5.9 ± 1.2 **

Figure 1. Expression of AT1 receptor in renal distal tubular cells. Immunohistochemical reaction, x200.

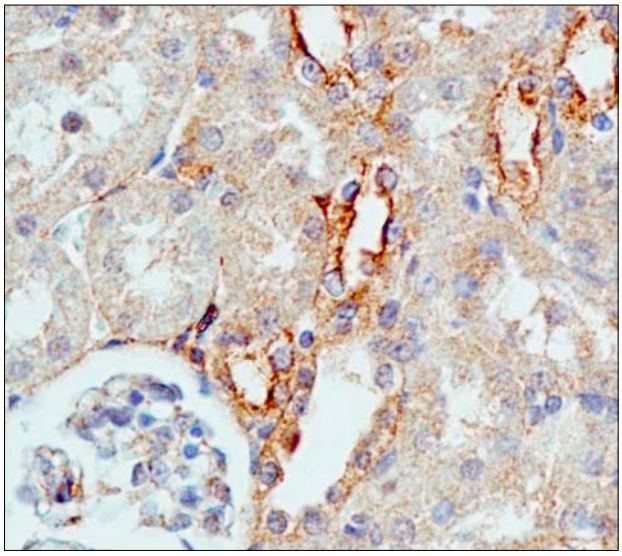
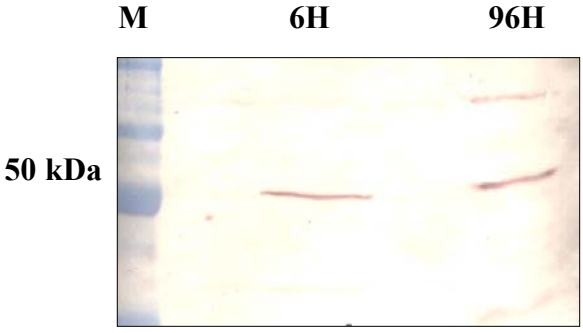


Figure 2. Expression of AT1 receptor protein in kidney cortex homogenate 6h and 96h after exercise. M - molecular marker. Western Blot analysis.



animals (N=6). The animals from the exercise group were subjected to running on a treadmill at 1.0 km/h until exhaustion. After the exercise, the animals returned to their cages and were randomly grouped into animals, killed after 6 hrs (N=6) from the exercise cessation and those, killed after 96 hrs (N=6) from the same time point. Control animals (N=6) remained in their cages throughout the experiment. All the animals were anaesthetized and decapitated. Two kidneys were excised from each rat. The right kidney was fixed in 4% buffered formaldehyde solution for 24 hrs and embedded in paraffin. The left kidney was frozen in liquid nitrogen and stored at -80^{0}C. In paraffin sections, apoptosis was detected by the TUNEL technique, using the ApopTag® Plus Peroxidase In Situ Apoptosis Detection Kit (INTERGEN, Norcross, USA). All the immunocytochemical reactions were performed in paraffin sections. The expression of AT1 and AT2 receptors was demonstrated, using mouse monoclonal antibodies (dilution: 1:200, Santa Cruz Biotechnology, Santa Cruz, CA, USA). All the reactions were accompanied by negative controls in which specific antibodies were substituted by the Primary Negative Control reagent. The investigated antigens were visualised, using biotinylated antibodies and streptavidin-biotinylated peroxidase and diaminobenzidine (LSAB2 kit and DAB, DakoCytomation, Denmark). AT1 receptor protein expression was assessed by the Western blot technique. One block of tissue from each rat was homogenized in a Tris-EDTA buffer. Crude membrane fractions were separated by SDS-PAGE and were electrotransferred to supported nitrocellulose. AT1 receptor protein bands were detected, using a primary antibody (1:500 dilution) from the Santa Cruz Biotechnology (Santa Cruz, CA, USA) and to develop Western blots, we used the chromogenic substrate - BCIP/NBT. Statistical analysis of the results was conducted by using the Chi-square test and the Statistica 5.1 PL software (StatSoft, Cracow, Poland). The differences were considered significant if $p < 0.05$.

Results

A significant increase was observed in the number of apoptotic nuclei in renal tubular cells of all the exercised animals, in comparison to that in the sedentary group (Tab. 1). The distal convoluted tubules displayed a strong expression of both angiotensin AT1 and AT2 receptors after 6, as well as after 96 hours from the exercise (Fig. 1). The obtained results were confirmed by Western blot, which revealed an increased expression of AT1 and AT2 receptor proteins (Fig. 2).

Discussion

It is well known that acute exercise can cause skeletal muscle damage, including apoptosis of myonuclei [4]. The generation of reactive oxygen species (ROS) after an intensive exercise seems to be responsible for exercise-induced changes in many organs. Our previous studies have demonstrated the presence of apoptosis in rat renal tubular cells after excessive exercise; however, the induction of apoptosis in kidney tubular cells was thought to be not associated with oxidative stress [3]. Recent studies suggest that angiotensin II plays a prominent role in the progression of renal injury. Although it was

assumed that angiotensin II stimulates cell proliferation via AT1 receptor and apoptosis via AT2 receptor [5], many authors have recently suggested that both AT1 and AT2 receptors influence the apoptotic process in the kidney [6]. One of the proposed mechanisms of apoptosis induction in kidney mesangial cells damage is the oxidative stress in response to stimulation of AT1 and AT2 receptors [7]. In the present study, we demonstrated an increased expression of AT1 and AT2 receptors in distal renal tubular cells of all the exercised animals. Moreover, there was a strong correlation between the occurrence of apoptosis and the increased expression of AT1 and AT2 receptors only in the cells of distal convoluted tubuli in rat kidneys. Conversely, Bhaskaran et al. [8] demonstrated that angiotensin II promoted apoptosis of renal proximal tubular cells via both AT1 and AT2 receptors *in vitro* and that the angiotensin-induced apoptotic process is mediated through oxidative stress. Nevertheless, we demonstrated in the previous work [3] that an induction of apoptosis in kidney tubular cells was thought to be not associated with the oxidative stress. The different distribution of apoptotic process in renal tubular cells probably depends on the nature of the triggering factor (e.g., oxidative stress, binding of apoptotic factors, DNA damage). We presume that exercise-induced apoptosis of kidney tubular cells could be mediated by both angiotensin AT1 and AT2 receptors, which are expressed in distal tubular cells. The stimulation of both AT1 and AT2 receptors could be associated with an increased expression of angiotensin-induced upregulation of p53, rather than with angiotensin-induced oxidative stress.

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