Changes in proteasome activity in the ischemic kidney of rat with experimental renovascular hypertension

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Abstract

A long-lasting renal ischemia, followed by the left renal artery clipping (two-kidney, one clip Goldblatt model in rats) led to a marked decrease in proteasome chymotrypsin-like activity in the ischemic kidney. This activity was, however, significantly raised upon the stimulation with an artificial 20S proteasome activator SDS (0.025%). No changes were observed in either the levels of the constitutive 20S proteasome subunit (α5) or of its protein activator, PA28α, in the kidneys by Western blot. These preliminary results indicate that an inhibition of proteasome activity may result from a dissociation of the active proteasome complexes into the inactive 20S proteasome and its endogenous activators after a long-lasting renal ischemia.

Key words: proteasome, renal ischemia, rat.

Introduction

The proteasome-dependent proteolytic pathway is responsible for the degradation of damaged proteins, and many short-life regulatory proteins, involved in inflammatory processes, cell cycle regulation and gene expression [1, 2, 3]. In the living cell, proteasomes are present in a latent form (20S proteasome, 700kDa) and in active complexes with the protein regulators: PA28 and PA700 [4, 5]. PA700 enhances the ability of 20S proteasome to degrade the ubiquitinated proteins in an ATP-dependent manner, whereas PA28 regulator, in association with 20S proteasome, stimulates its capacity to the generation of antigen peptides, presented to the immune system on MHC class I molecules [1].

Alterations in proteasome activity have been demonstrated in a large number of pathological conditions, including cancer, neurodegenerative disorders, acute wasting conditions, ischemia-reperfusion injury and ischemic acute renal failure [3, 6, 7, 8]. In the present study, we investigated, whether a long-lasting renal ischemia, followed by the left renal artery clipping (two-kidney, one clip Godblatt model in rats), could influence the proteasome activity in the kidneys.

Material and methods

All the experimental procedures, involving animals and their care, were conducted in conformity with the national and international laws and with the Guidelines for the Use of Animals in Biochemical Research. Male Wistar rats (n=5), weighing 160 - 180 g each, were anesthetized with pentobarbital (40 mg/kg, i.p.). Two-kidney one clip (2K-1C) renovascular hypertension was induced by a partial, standardized clipping of the left renal artery (0.2-mm internal diameter) for 6 weeks [9]. Rat kidneys (unclipped and clipped) were placed in 50mmol/L Tris-HCl buffer at pH 8.0, containing a protease inhibitor cocktail, and were homogenized in ice with a Potter-Elvehjem homogenizer. The homogenates were then centrifuged for 60 minutes at 15000 x g +4^{0}C. The protein concentration of the supernatants was determined by the method of Bradford [10], using the Bio-Rad assay reagent with bovine serum albumin as a standard. The proteasome activity in kidney extracts (20µg of protein) was measured, using fluorogenic peptide Suc-Leu-Leu-Val-Tyr-AMC (100µmol/L) (Affinity, UK) in a 50mmol/L Tris-HCl buffer (pH 7.5), containing 5mmol/L EDTA, 1.0 mmol/L ATP and 15% glycerol, in either the absence or the presence of 20S proteasome activator 0.025% SDS [11]. For Western blot analysis, aliquots of the homogenates (20µg of protein per line) were

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Figure 1. Effects of left renal artery clipping on the chymotrypsin-like activity of proteasome in either the absence or the presence of SDS in clipped and unclipped rat kidneys.

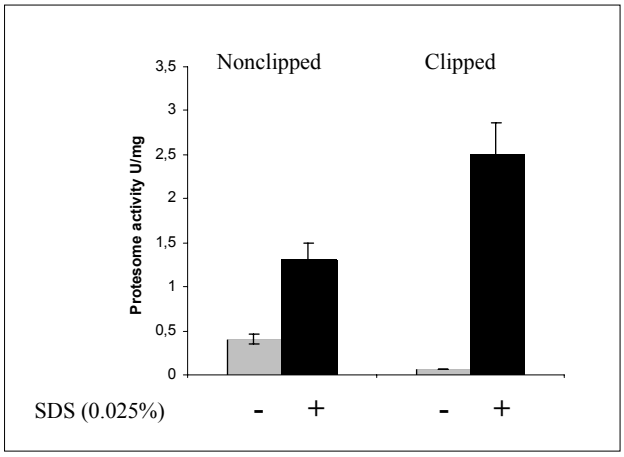
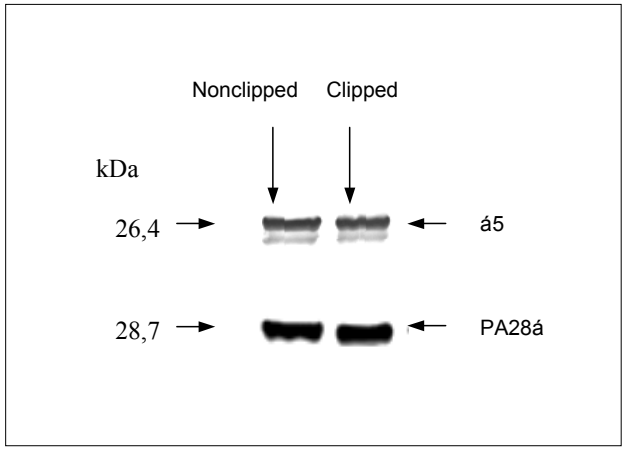


Figure 2. Effects of left artery clipping on the levels of constitutive proteasome subunit α5 and proteasome activator PA28α in clipped and unclipped rat kidneys.



electrophoresed by SDS-PAGE on 10% gels under reducing conditions, according to Laemmli [12], and then the proteins were electroblotted onto nitrocellulose. The nitrocelluloses were incubated with anti-α5 (20S proteasome) and anti-PA28α antibodies (Affinity, UK). The proteasome subunits were identified, using the alkaline phosphate-conjugated anti-rabbit IgG as secondary antibody and visualized, using phosphatase substrate (Sigma, USA). The results are given as means ±SE of three independent measurements. Any value of $P<0.05$ was considered significant.

Results

The left renal artery clipping, lasting six weeks, led to a significant reduction in the clipped kidney mass (0,16g ±0,07), and statistically significant increases in the contralateral kidney mass, compared to respective values in the controls (1,35g ±0,13).

Proteasome activity, measured by the use of Suc-Leu-Leu-Val-Tyr-AMC substrate, clearly decreased in the clipped kidney, compared to that in the unclipped organ (*Fig. 1*). After the addition of 0.025% SDS, which is non-physiological activator of the latent 20S proteasome, that activity was significantly raised in the ischemic kidney (about 18 times).

No changes in the levels of the constitutive 20S proteasome subunit α5 were detected by Western blot in either the clipped or the non-clipped kidneys (*Fig. 2*). Likewise, the proteasome activator PA28α subunit was expressed at equally high levels in both kidneys.

Discussion

In the present study, we demonstrated that a long-lasting renal ischemia, followed by the left renal artery clipping, lead to a significant inhibition of proteasome activity in the ischemic kidney. As demonstrated by the Western blot analysis, the inhibition of the activity was not, however, accompanied by any reduction in 20S proteasome and its protein activator PA28 levels. Therefore, these data, together with the fact, that proteasome activity was significantly raised upon the addition of 0.025% SDS, a potent artificial activator of the latent 20S proteasome, indicate that the loss in proteasomal activity in the ischemic kidney could be due to irreversible dissociation of the active proteasome complexes into an inactive free 20S proteasome and its protein activators.

Ischemia leads to complex cellular events, which can affect proteasome function, including depletion of ATP and disturbances of intracellular calcium haemostasis. Several *in vitro* studies have demonstrated that ATP depletion causes an almost complete dissociation of active PA700-20S-PA700 and PA700-20S-PA28 complexes [4, 5, 13]. It has also been demonstrated that the binding of calcium ions to proteasome regulator PA28 inhibits the peptidase activity of PA28-20S proteasome complexes [14]. Therefore, it is likely that suppression of proteasome activity in the ischemic kidney may result from disturbances in the formation of active proteasome complexes, due to the depletion of ATP and/or increase in intracellular calcium levels during a long-lasting renal ischemia.

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