Ca-Dependent ATPase Activity and Lipid Peroxidation in the Microsome Fraction of Renal Medulla after Thermal Ischemia with and without α-Tocopherol Protection

E. A. Golod and M. I. Savina

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Thermal ischemia of rat kidneys for 30 min induces lipid peroxidation with simultaneous activation of Ca-dependent ATPase in microsomes. In the presence of the channel-forming antibiotic alamethicin this activity decreases, indicating an increased passive permeability of the membrane vesicles for Ca^{2+} and ATP with a decrease in the true activity of the enzyme after ischemia. α-Tocopherol reduces the intensity of lipid peroxidation in the microsome fraction isolated from kidneys after thermal ischemia and protects ATPase activity in the presence and absence of alamethicin in the incubation medium. It is suggested that peroxidation of membrane phospholipids activated by thermal ischemia is one of the causes of decreased true activity of Ca-dependent ATPase and increased passive permeability of the kidney membranes for Ca^{2+} and ATP.

Key Words: *ischemia; microsomes; peroxidation; Ca-ATPase; α-tocopherol*

Activation of lipid peroxidation (LPO), a universal mechanism underlying membrane damage, is a pathogenic factor of tissue ischemia [1,3,5]. Activation of LPO in ischemia may lead to membrane destruction, thus impairing the function of membrane-bound transport systems, including Ca pumps [2,9,10].

It was reported that the antioxidant α-tocopherol (TPH) not only prevents accumulation of LPO products in biological membranes but also protects calcium transport [2]. The functional state of Ca pump in the cell can be estimated from the activity of Ca-dependent ATPase in isolated membrane fractions, specifically, in microsomes. However, this is a methodically complex approach, since the permeability of microsomal membranes for Ca and ATP is very low and the active centers of enzymes are oriented into microsome [4,7].

Institute of Urology, Ministry of Health; Russian State Medical University, Moscow

Alamethicin, an antibiotic capable of forming membrane channels and thus increasing the membrane permeability for Ca^{2+} and ATP was employed to assay the true activity of microsomal Ca-dependent ATPase [4]. The maximum activity of the enzyme in microsomal fraction isolated from rat kidney medulla was observed at an alamethicin concentration of 20-30 μg/ml.

In the present study we evaluated the role of LPO activation caused by thermal ischemia on Ca-dependent ATPase activity in rat kidney medulla microsomes in the presence and absence of alamethicin and examined the protective effect of TPH on this activity.

MATERIALS AND METHODS

Microsomes were isolated from renal medulla of 44 male rats weighing 180-200 g. The content of malonic dialdehyde (MDA) and the activity of Ca-dependent ATPase were determined. Twenty four rats (12

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TABLE 1. MDA Content and Ca-Dependent ATPase Activity in Microsomes Isolated from Kidneys of Intact Rats and Rats Subjected to Thermal Ischemia with and without α-Tocopherol Protection (*M±m*)

Parameter	Microsomes from rat kidneys			
	intact		after thermal ischemia	
	without protection (*n*=12)	after protection (*n*=10)	without protection (*n*=12)	after protection (*n*=10)
MDA content, nmol/mg protein	0.631±0.018	0.601±0.014	0.961±0.078*	0.701±0.021***
Activity of Ca-dependent ATPase, μmol P/min/mg protein:				
without alamethicin	0.56±0.042	0.55±0.035	0.88±0.060*	0.68±0.021***
with alamethicin	1.88±0.021	1.88±0.016	1.38±0.040*	1.63±0.032**

Note. *p<0.001, **p<0.02 compared with the control without protection; *p<0.001 compared with the data obtained without protection.

after thermal ischemia of both kidneys and 12 intact) were not treated and 20 rats (10 after thermal ischemia of both kidneys and 10 intact) were injected with TPH (30% solution, 5 mg/100 g intramuscularly) 24 and 3-4 h before experiment. For thermal ischemia of kidneys, the abdominal cavity was opened, and the vascular stems of both kidneys were clamped for 30 min. Both kidneys were extirpated after 30 min. The medulla from ischemic and intact kidneys was isolated on ice and homogenized in medium containing 0.32 M sucrose, 5 mM Tris-buffer, 10 mM histidine (pH 7.4) at 4°C. Microsomes were pelleted by centrifugation of postmitochondrial supernatant at 70,000*g* for 60 min and used in experiments. The activity of Ca-dependent ATPase was determined as the difference between ATPase activity in calcium-containing (100 mM KCl, 4 mM $MgCl_2$, 3 mM ATP, 40 μM $CaCl_2$, 5 mM NaN_3, 0.5 mM ouabain, 10 mM imidazole, pH 7.2 at 37°C) and calcium-free medium (0.5 mM EGTA instead of $CaCl_2$). The microsomes (80-100 μg protein/ml) were incubated for 1 min at 37°C in 1 ml medium with constant stirring, and the reaction was stopped by the addition of 20% trichloroacetic acid (1 ml). In the true Ca-dependent ATPase activity assay, alamethicin in 60% ethanol was added before microsomes, and the reaction was initiated by the addition of ATP. The ATP hydrolysis was assessed by measuring the inorganic phosphorus (P) concentration in the incubation medium [8].

The intensity of LPO in microsomes was estimated by MDA formation in the presence of thiobarbituric acid [6] with the coefficient of extinction equal to 1.56×10^5 $M^{-1}cm^{-1}$. The protein content in the microsomal fraction was determined by the biuret method.

RESULTS

In the microsomes isolated from intact kidneys, Ca-dependent ATPase activity was 0.56±0.042 μmol P/min/mg protein in the absence of alamethicin, increasing to 1.88±0.02 μmol P/min/mg protein in its presence. This increase can be attributed to higher permeability of microsomal membranes for Ca^{2+} and ATP after the addition of the antibiotic. This prevented inhibition of Ca-ATPase by high Ca^{2+} concentrations and provided the transport of ATP to ATPase molecules with active centers oriented into the vesicle [4]. In the absence of alamethicin, Ca-dependent ATPase activity in microsomes isolated from ischemized kidneys was 0.88±0.06 μmol P/min/mg protein, which was significantly higher than in microsomes from intact kidneys (p<0.001). In the presence of alamethicin, the enzyme activity of microsomes from ischemized kidneys was increased to a much lesser degree than in microsomes from intact kidneys (p<0.001, Table 1). The increase in the microsomal activity of Ca-dependent ATPase in alamethicin-free medium and the decrease in this activity in the presence of alamethicin points to a rise of the microsomal membrane permeability for calcium in ischemia. An increase in membrane permeability caused by ischemia provides more access for Ca^{2+} and ATP to the active centers oriented into microsomes and lowers intramicrosomal concentration of calcium ions which inhibit Ca pump [4]. We have hypothesized that impaired membrane permeability and a decrease in the true Ca-ATPase activity in ischemia are associated with activation of LPO, since LPO is known to affect the barrier function of membranes and inhibit membrane-bound enzymes [2,9]. The MDA concentration increased in microsomes isolated from ischemized kidneys (Table 1), suggesting that LPO contributes to ischemia-induced modifications of Ca-dependent ATPase activity. To clarify this issue, we performed a series of experiments with TPH. In media with and without alamethicin we observed no statistically significant changes in MDA content and Ca-ATPase activity in micro-

somes isolated from ischemized kidneys of TPH-treated rats (Table 1). In the absence of alamethicin, these parameters were significantly lower in ischemized kidneys from TPH-treated rats than in microsomes from untreated rats subjected to ischemia, while the true Ca-ATPase activity was higher (Table 1).

Our results indicate that peroxidation of membrane phospholipids contributes to a decrease in the activity of Ca-dependent ATPase and to the ischemia-induced increase in the permeability of renal membranes for Ca^{2+} and ATP.

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