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Short communication

Na^+,K^+-ATPase activity of rabbit kidney cortex membranes in ischemia and reperfusion

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The activity of Na^+,K^+-ATPase in the microsomal fraction of rabbit kidney cortex was strongly decreased by ischemia and increased slightly, but not significantly, after reperfusion. These changes were correlated with a dramatic increase in lipid peroxidation in microsomes isolated from both ischemic and reperfused kidneys. This correlation may point to irreversible impairment of the enzymatic function under the influence of either oxygen free radicals or lipid peroxidation.

Tissue injury caused by ischemia and reperfusion is a current topic in biochemical research [1 - 3]. The role of free radicals in the ischemic process has been implicated in a number of human diseases involving lung, kidney and the central nervous system [2, 4, 5]. Investigations have been carried out [3, 6] to evaluate protective agents against various ischemic diseases associated with the formation of oxygen free radicals: the superoxide anion $O_2^{\bullet}$ and the hydroxyl radical $OH^{\bullet}$. The latter is formed when the superoxide anion reacts with hydrogen peroxide in the presence of transition ions in the so-called Fenton reaction [3, 7]. Hydroxyl radical is one of the most reactive species known in organic chemistry, capable of reacting with almost every organic compound present in a living cell [8]. Once these oxygen intermediates are formed in large quantities, a number of destructive chain reactions can occur leading to cell death. Free radicals can attack polyunsaturated chains of fatty acid moieties of membrane lipids and initiate the process of lipid peroxidation. Lipid peroxides are, in turn, powerful inhibitors of numerous enzymes and can also decompose to yield products that damage both proteins and cell membranes [3, 7, 8].

A number of low molecular weight compounds that can produce superoxide anion are present in the cytosol of living cells. Among them are thiols, hydroquinones, catecholamines and flavines. There are also cytoplasmic enzymes capable of generating free radicals as, for example, xanthine oxidase which increases its activity in ischemia through the catabolism of ATP [8].

There is ample evidence that much of tissue injury is caused be the re-flow of oxygenated blood after ischemia [9]. The cells are rich in both xanthine oxidase, formed from xanthine dehydrogenase during ischemia, and its substrate. It is likely that, with the increased oxygen supply after reperfusion, superoxide anions are formed in large quantities, leading to extensive cell injury [1, 9].

The aim of the present work is to evaluate whether lipid peroxidation *in vivo* can affect the membrane-bound enzyme, Na^+,K^+-ATPase. This knowledge would be important for evaluation of the extent of kidney dysfunction caused by ischemia and reperfusion, in particular when the organ is preserved outside the body during transplantation.

MATERIALS AND METHODS

Twenty male rabbits about 2 months old (body weight 1.5 - 2.5 kg) kept on a standard laboratory diet were used. All animals were divided into two groups: 10 controls and 10 subjected to kidney ischemia. They were deprived of food but had free access to water 12 h preoperatively. Anaesthesia was applied by intramuscular injection of a mixture of 0.3 ml Xelazine and 0.5 ml 2% Xylazine (Bayer) per kg body weight. The same mixture was injected at intervals of 30 min to maintain anaesthesia during the operation. The rabbits breathed room air spontaneously during the operation, and the blood loss and perspiration was compensated by giving isotonic saline intravenously. The abdominal cavity was exposed through a longitudinal middle line incision and both kidney arteries were clamped for 60 min. Thereafter, the left (ischemic) kidney was excised and to the right one blood circulation was restored by removing the clam. This kidney was excised after 10 min of reperfusion. One of the kidneys of the control animals was removed under sterile conditions.

The excised kidneys were decapsulated and the outermost slices of the cortex were homogenized at 4°C by eight strokes of a teflon pestle at 2500 rev./min. For lipid peroxide analysis, 10% homogenate was prepared in 1.15% KCl according to Uchiyama & Milhara [9]. For isolation of cortex membranes, the tissue was homogenized in the medium containing 250 mM sucrose, 20 mM Tris/HCl (pH 7.2), 0.5 mM dithiothreitol and 0.2 mM phenylmethylsulphonylfluoride. The isolation was performed according to Marin *et al.* [10]. The homogenate was centrifuged at 1000 × *g* for 10 min. The pellet was resuspended in the homogenization medium and centrifuged again. Both supernatants were combined and centrifuged at 9500 × *g* for 10 min. The supernatant and the soft light upper portion of the pellet were combined and centrifuged at 48000 × *g* for 20 min. The upper layer of the resulting pellet containing the cortex membranes was collected.

Lipid peroxidation was measured by determining formation of malondialdehyde as described by Uchiyama & Milhara [9].

Na^+,K^+-ATPase was assayed by the method proposed by Serpersu & Ciliv [11]. The medium contained 50 mM NaCl, 20 mM KCl, 3 mM $MgCl_2$, 100 mM Tris/HCl (pH 7.4) and 3 mM ATP. The reaction was started by addition of the membrane suspension and the mixture was incubated for 60 min at 37°C. The reaction was stopped by adding cold 10% trichloroacetic acid, the precipitated protein was removed by centrifugation, and inorganic phosphate formed was analyzed according to Ames [12]. The activity of Na^+,K^+-ATPase was calculated as the difference between the total activity and that in the presence of 1 mM ouabain.

Protein was determined by the method of Lowry *et al.* [13].

Statistical determinations were done by Minitab statistical programme run by Amstrad personal computer. The results are expressed as means ± S.D.

RESULTS AND DISCUSSION

As shown in Table 1, ischemia resulted in an almost fivefold increase in lipid peroxidation. This was further potentiated by reperfusion. Both increases are statistically significant ($P < 0.05$). Ischemia caused a decrease in Na^+,K^+-ATPase activity to one fourth ($P < 0.05$) and reperfusion produced a slight increase with respect to ischemia. The latter change, however, was not statistically significant ($P > 0.05$).

It is well known that Na^+ transport in kidney nephrons utilizes ATP. In proximal tubules Na^+ enters the cytoplasm in a co-transport with other solutes like glucose, amino acids and phosphate [14]. The Na^+,K^+-pump is also effective in maintaining the osmotic gradient of the cell and controls the cell volume [14]. Studying the effect of oxygen metabolites on the cardiac Na^+,K^+-pump, Xie *et al.* [15] reported an inhibition of Na^+,K^+-ATPase by incubation with xanthine and xanthine oxidase.

Ischemia and reperfusion have a stimulatory effect on the release of fatty acids, in particular arachidonic acid, possibly caused by activation of phospholipase A_2 due to the increase in intracellular level of Ca^{2+} during ischemia. These fatty acids can inhibit Na^+,K^+-ATPase and, in addition, stimulate the cyclooxygenase path-

Table 1

Effect of kidney ischemia and reperfusion on lipid peroxidation and Na^+,K^+-ATPase activity in kidney cortex membranes

Lipid peroxidation was expressed as the amount of malondialdehyde formed and Na^+,K^+-ATPase activity as inorganic phosphate liberated. All values are means for 10 animals ± S.D.

	Lipid peroxidation (nmol/mg protein)	Na^+,K^+-ATPase activity (μmol/h per mg protein)
Control	1.14 ± 0.29	0.524 ± 0.056
Ischemia	5.33 ± 1.15	0.138 ± 0.060
Reperfusion	6.89 ± 1.13	0.149 ± 0.047

way which promotes synthesis of prostaglandin F_2 and thromboxane A_2 [16]. Lack of oxygen supply followed by reoxygenation results in structural damage of both lipid and protein components of the cell [17]. Lipid peroxidation alters membrane permeability and may lead to structural or functional damage of the Na^+-pump, resulting in an imbalance of Na^+, K^+ and Cl^- between the cell and its surrounding medium. As a result, the plasma membrane potential and cell osmolarity are altered, resulting in cell shrinkage. The present investigation shows that ischemia produces lipid peroxidation in kidney membranes which is further potentiated by reperfusion. These changes are correlated with a dramatic decrease of Na^+,K^+-ATPase activity, and it can be deduced that the Na^+,K^+-pump is inhibited as well. It is, however, not clear whether this inhibition is produced by lipid peroxides or directly by oxygen free radicals.

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