Effects of theophylline, verapamil, and mannitol on oxygen consumption of ischemic rat kidneys

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Abstract

This study was designed to determine, through the measurement of oxygen consumption (Qo2), whether pretreatment and posttreatment with theophylline, verapamil, and mannitol provide protection from damage in the cortex and medulla of rat kidneys subjected to 60 and 120 minutes of ischemia. Qo2 levels in both tissues were examined 1 day after each ischemia. After 60 minutes of ischemia, pretreatment with theophylline and verapamil primarily provided protection from ischemic change in cortical Qo2. Pretreatment with mannitol provided protection from both cortical and medullary changes in Qo2, but posttreatment with mannitol only protected cortical Qo2. After 120 minutes of ischemia, only pretreatment with verapamil was effective in protecting cortical Qo2 from ischemic injury. These observations demonstrate that theophylline, verapamil, and mannitol are effective in protecting renal Qo2 in the ischemic kidney. During this study, it became clear that there were differences in the effectiveness of theophylline and verapamil on Qo2 in the ischemic kidney between pretreatment and posttreatment, and also between the cortex and the medulla.

Introduction

Acute renal failure (ARF) is a clinical syndrome characterized by a rapid deterioration in renal function. Renal ischemia is the most common cause of ARF. This condition frequently progresses to renal insufficiency or renal failure. If a given situation of ARF is adequately treated, renal function can be relatively well preserved. Therefore, attempts have been made to investigate the efficacy of various treatments in improving the condition of ARF. It was reported recently that theophylline [1, 2], a potent adenosine receptor antagonist, verapamil [3-6], a potent calcium channel blocker, and mannitol [7-9] were effective in improving damaged renal functions of the ischemic kidney. However, the protective effects of individual agents on the ischemic kidney show appreciable differences.

The present study was designed to investigate the existence of differences in the effectiveness of theophylline, verapamil, and mannitol in the protection of oxygen consumption (Qo2) in the ischemic rat kidney between pretreatment and posttreatment, and also between the cortex and the medulla.

Materials and Methods

Adult male Wistar rats, weighing between 320 and 500 grams, were used as the subjects of this study. To induce renal ischemia, the experimental rats were anesthetized with sodium pentobarbital (50 mg/kg body weight) administered intraperitoneally (i.p.). A right dorsal incision was made to expose the renal vessels of the right kidney. The artery was carefully separated from the vein to avoid damaging the nerve plexus. Heparin (50 U/kg body weight) was injected via the tail vein to prevent coagulation during occlusion. Approximately 5 minutes later, the right renal artery was occluded without interfering with the vein, by a smooth surfaced miniclamp for 60 or 120 minutes. Only kidneys exhibiting an immediate paling of the whole surface were used for the experiments. After each ischemic period, the arterial clamp was removed. Resumption of the blood flow was con-
firmed by direct observation of a color change of the kidney surface. The right dorsal incision was then closed with sutures. The animals were allowed to recover in their cage where they were provided with food and water ad libitum.

A continuous intravenous infusion of saline alone, or theophylline (12.5 mg/kg body weight prime followed by a sustaining dose of 0.125 mg/kg/minute) in saline, or verapamil (5 µg/kg/minute) in saline, or 5% isotonic mannitol was begun via the femoral vein at the speed of 0.055 ml/minute for 60 minutes using an infusion pump (TR-20B, Toray Co., Japan) before (pretreatment) or after (posttreatment) ischemia for either 60 or 120 minutes.

One day after the ischemic operation, the rats were anesthetized with i.p. sodium pentobarbital (50 mg/kg body weight). A midline abdominal incision was made immediately to expose the right ischemic kidney. The right kidney was carefully separated from the surrounding adipose tissues. After ligation of the right renal artery and vein, the right kidney was carefully removed. This kidney was cut into slabs, then the cortex and the medulla were carefully trimmed off with a sharp knife as quickly as possible. These dissected samples were further cut into 200-µm slices using a microslicer (DTK-1000, D.S.K. Co., Japan). These sliced samples were then incubated in the incubation solution until the Qo2 levels were measured.

In all aspects of the experiments, the rats were handled in accordance with the guidelines for the care and use of animals established by Niigata University.

**Measurement of Qo2**

A Clark-type oxygen electrode, designed and constructed for small samples (Rank Brothers Co., Cambridge, England) was used to measure Qo2. The tissue samples, the cortex (20–30 mg dry weight) and the medulla (10–20 mg dry weight), were respectively transferred into incubation chambers filled with the incubation solution (700–800 µl). The incubation solution was kept at 37.0°C and saturated with a mixed gas consisting of 5% CO2 and 95% O2 (pH: 7.44±0.016, PaO2: 603.4±20.3 mmHg, PaCO2: 36.0±1.1 mmHg; mean±SEM, n=10). The solution and the electrode were separated with a Teflon® membrane. A small stirrer was used during the measurements of Qo2 for about 5 minutes. The temperature in the small vessel remained nearly constant because of the use of the small stirrer and the short measuring time. After sealing the chamber, electrode output (mV) changed immediately from a relatively steady state to a rapidly decreasing state. The output was continuously recorded with a high-sensitivity pen recorder (VP-6610A type, Matsushita Co., Japan). Qo2 was calculated from change in the potential difference (PD, mV) during the initial 1 minute (delta PD) using the following equation:

\[ Qo2 = \text{delta PD}/\text{PD} \times 23.0 \text{ V/W} \]

in which PD is the initial output of the oxygen electrode without the sample when the incubation solution is saturated with a mixed gas of 5% CO2 and 95% O2, at 37.0°C. The figure, 23.0, is the coefficient of solubilized oxygen per ml H2O (µl O2/ml H2O) saturated with a mixed gas at 37.0°C. V is the fluid volume inside the vessel, and W is the dry weight of the tissue samples. The value of Qo2 was given in units of nl·min⁻¹·mg⁻¹ dry weight (d.wt.). The dry weight of each sample was measured after dessication at 150°C for 1.5 hours [10].

**Solution**

The incubation solution, a Krebs-Ringer-Henseleit solution, had the following composition (mM): NaCl, 123; KCl, 3.8; CaCl2·2H2O, 1.7; KH2PO4, 1.2; MgSO4·7H2O, 1.2; NaHCO3, 25; and D-glucose, 5.6, aerated with a mixed gas of 5% CO2 and 95% O2.

**Statistical evaluations**

Statistical analyses were performed using analysis of variance. A probability of less than 0.05 was considered significant. All data were expressed as the mean±SEM.

**Results**

**Table 1** shows the effects of pretreatment with theophylline, verapamil, and mannitol on the cortical and medullary Qo2 of the kidneys injured by 60 minutes of ischemia. The cortical Qo2 levels of each pretreatment were significantly higher than that of kidneys pretreated with saline alone. Moreover, in the medulla, only the Qo2 level of kidneys pretreated with mannitol was significantly higher than that of kidneys pretreated with saline alone.

**Table 2** shows the effects of each posttreatment on the cortical and medullary Qo2 of the kidneys injured by 60 minutes of ischemia. Only the cortical Qo2 level
of kidneys posttreated with mannitol was significantly higher than that of kidneys posttreated with saline alone. Posttreatment with theophylline, verapamil, and mannitol had no effects on the medullary Q\textsubscript{o2}.

**Table 3** shows the effects of each pretreatment on the cortical and medullary Q\textsubscript{o2} of the kidneys injured by 120 minutes of ischemia. Only the cortical Q\textsubscript{o2} level of kidneys pretreated with verapamil was significantly higher than that of kidneys pretreated with saline alone. Pretreatment with theophylline, verapamil, and mannitol had no effects on the medullary Q\textsubscript{o2}.

Table 3 summarizes the effects of each posttreatment on the cortical and medullary Q\textsubscript{o2} of the kidneys injured by 120 minutes of ischemia. Each posttreatment was ineffective in providing protection from changes in the cortical and medullary Q\textsubscript{o2} after ischemic injury.

**Discussion**

It is known that ischemia leads to an increase in the
tissue level of adenosine. The production of adenosine has been reported to occur primarily in the cortex, specifically at the level of the proximal tubule cells [11], ascending limb cells, and macula densa cells [12]. Adenosine has vasoconstricting actions and decreases the glomerular filtration rate (GFR). Accordingly, adenosine has a prominent role in the local control of glomerular hemodynamics [12]. Recently, it has been reported that pretreatment with theophylline is effective in increasing both the renal blood flow (RBF) and GFR in the initiation phase after 20 to 45 minutes of ischemia [1, 2], thus antagonizing the effects caused by adenosine. These previous reports suggested that the site of theophylline action was primarily in the cortex. The present study affirms these previous reports and demonstrates that pretreatment with theophylline effectively protects cortical QO2 from ischemic injury.

Concerning the effects of verapamil on ischemia, there have been several reports that pretreatment [3, 4] or posttreatment [4-6] was effective in protecting RBF and GFR when renal ischemia was induced by renal artery occlusion. The present study showed that verapamil was effective when it was given before ischemia. In posttreatment, however, verapamil exhibited no effect on either cortical or medullary QO2. In this regard, Burke et al. [4] demonstrated that a longer duration of verapamil infusion was necessary for the agent to have any effect when initiated after rather than before the renal ischemia. Therefore, the effects of verapamil might be related to the duration of the infusion before and after ischemia. In the present study, the duration of verapamil infusion was the same before and after ischemia. This might have been the reason why posttreatment with verapamil failed to show any protective effects on the damaged QO2 of the ischemic kidney. Moreover, our results showed that verapamil primarily protected cortical QO2 from ischemic injury. Other experiments have demonstrated that verapamil blocks the decrease in glomerular capillary permeability on account of contraction of glomerular mesangial cells [13, 14]. A profound and significant increase was observed in calcium intake after in the proximal segments 30 minutes of anoxia, but this was prevented by verapamil [15]. These findings suggest that the primary site of action of verapamil is in the cortex. This is consistent with the present results.

In several experiments in which mannitol was used, it has been demonstrated that pretreatment with isotonic mannitol protected both RBF and GFR against ischemic injury [7, 8]. Fuenzalida et al. [9] also reported that posttreatment with mannitol restored GFR in ARF induced by partial occlusion of the aortic artery for 30 minutes. However, it was not effective after prolonged hypoperfusion for more than 120 minutes. In our results, mannitol which was given before ischemia for 60 minutes was effective in protecting cortical and medullary QO2. Additionally, mannitol given after ischemia was also effective in protecting cortical QO2 of the ischemic kidney. It became apparent in the damaged QO2 of the ischemic kidney, therefore, that mannitol had both prophylactic and therapeutic effects in the case of ischemic injury, as previously noted by Levinsky [16]. However, it was not effective with 120 minutes of ischemia. These results are consistent with the previous observations noted by Fuenzalida et al. [9].

It is well known that renal QO2 is tightly coupled with transporting functions, including active sodium reabsorption [17, 18]. Therefore, we believe that the protection provided by theophylline, verapamil, and mannitol in cortical and medullary QO2 of the ischemic kidneys are effective in preserving renal functions after ischemic injury, although these agents differ in their effects on the damaged QO2 of the ischemic kidney.

In conclusion, the present result shows that pretreatment with theophylline and verapamil, and pre- and posttreatments with mannitol are effective in protecting renal QO2 from ischemic injury for up to 60 minutes. Furthermore, pretreatment with verapamil is also effective against ischemic injury for up to 120 minutes. In particular, theophylline and verapamil protect cortical QO2 of the ischemic kidney. In addition, clear differences have been observed in the effectiveness of theophylline and verapamil on QO2 in the ischemic kidney between pretreatment and posttreatment, and also between the cortex and the medulla.

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