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Effect of Renal Ischemia in Tetraethylammonium Transport in Rabbit Renal Coritcal Slices

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= ABSTRACT =

This study was carried out to determine effect of acute renal ischemia on transport function of organic cation, tetraethylammonium (TEA), in rabbit kidney proximal tubule. Clamping of the renal artery for 30 and 60 min produced a polyuria which was accompanied by an increase in Na^+ excretion. The capacity of kidney cortical slices to accumulate TEA was increased after 30 and 60 min of ischemia. When blood flow was restored for 30 min after 30 and 60 min of ischemia, the augmented TEA uptake was recovered to the control values. Oxygen consumption of cortical slices was stimulated after 30 min of ischemia, whereas it was not altered by 60 min of ischemia. A 90-min ischemia produced a significant inhibition of TEA uptake and tissue oxygen consumption. These results suggest that the basolateral transport system for organic cation persists after ischemic periods of 60 min despite evidence that tubular reabsorptive mechanism of Na^+ and water is markedly impaired. This may indicate that the active secretory systems of proximal tubule are more resistant to ischemic injury than the reabsorptive systems.

Key Words: Renal ischemia, Organic cation uptake, Oxygen consumption, Cortical slices, Rabbit proximal tubule.

INTRODUCTION

Acute renal failure produced by ischemia is a clinical and experimental syndrome characterized by a combination of renal vasoconstriction, renal tubular dysfunction, and fall in glomerular filtration rate (GFR) (Barnes et al, 1981; Donohoe et al, 1978; Finn, 1981). However, the mechanism (s) and cellular site (s) responsible for these changes remain to be explored.

The animal models of renal ischemia induced experimentally by clamping the renal artery have been largely used in an attempt to understand the pathophysiologic events involved in ischemic acute renal failure (Stein et al, 1978; Madias et al, 1988). In fact, pathophysiology of renal ischemia in man strongly resembles that seen in the experimental animals (Myers et al, 1984). Renal ischemia results in significant alterations in tubular cell function which are characterized by a marked reduction in tubular reabsorption of Na^+, water and glucose (Herminghuysen et al, 1985; Johnston et al, 1984; Molitoris & Kinne, 1987). However, the effect of ischemia on the active tubular secretion of organic compounds has not been systematically explored.

It has been reported that the glycerol-induced renal failure, which is considered to be mediated by renal ischemia, reduces the net secretion of organic cation (Shim et al, 1983; Lin & Lin, 1988). Berndt (1976), however, found that the accumulation of TEA was unaf-

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fected after 60 min ischemia in rat kidney. Thus, the alterations in the organic cation transport by renal ischemia have not been fully elucidated.

The present study was undertaken to characterize changes in the basolateral transport of organic cation in ischemic rabbit kidney. TEA uptake and oxygen consumption were measured in cortical slices of kidneys obatined immediately after ischemia or after 30 to 120 min of reflow following ischemia.

METHODS AND MATERIALS

In vivo experiments

New Zealand White rabbits of both sexes weighing 1.5-2.5 kg were used in all experiments. Anesthesia was induced by intramuscular injection of a mixture of ketamine (25 mg/kg) and xylazine (5 mg/kg) and was maintained by repeated small doses at intervals of approximately 30 min. After cannulation of the trachea, a polyethylene catheter was introduced into the carotid artery for recording of blood pressure. The ear vein was catheterized for infusion of solution. Isotonic saline (40 ml) was given intravenously to replace blood loss and perspiration during the operation. The abdomen was opened by a middle line incision. Heparin (4,000 IU) was given intravenously and both ureters were catheterized for urine collection. After completion of surgical procedures, saline was infused throughout the experiments at a rate of 0.5 ml/min.

Unilateral kidney ischemia was induced by complete occlusion of the left renal artery with arterial clamp and the right kidney served as a control kidney. The abdominal wall was then closed with forceps. After the indicated ischemic period, the ligature was released and the kidney was allowed to be recirculated for 2 h. Urine samples were collected separately from both ureters.

Urine samples were analyzed for Na^+, K^+ (flame photmeter, Beckman) and Cl^- (Chloridometer).

Slice experiments

Cortical slices were prepared and TEA uptake was measured as described previously (Kim et al, 1986, 1988). Following the ischemia or reflow, both kidneys were removed and the renal artery was immediately perfused with an ice-cold isotonic saline solution containing 140 mM NaCl, 10 mM KCl and 1.5 mM $CaCl_2$, to remove as much blood as possible. Thin (0.4-0.5 mm thick) slices of renal cortex were prepared using a Stadie-Riggs microtome and were stored in an ice-cold modified Cross-Taggart medium containing 130 mM NaCl, 10mM KCl, 1.5mM $CaCl_2$ and 20 mM Tris/KCl (pH 7.8). Approximately 50 mg (wet wt.) of slices were then transferred into a 20 ml beaker containing 4 ml of the modified Cross-Taggart medium, and incubated with 10 μM ^{14}C-TEA (Amersham, Alrington heights, IL). The incubation was carried out for 60 min in a Dubnoff metabolic shaker at 25°C under a 100% oxygen atmosphere.

Immediately after incubation, the slices were quickly removed from the beaker, blotted, weighed and solubilized in 1 N NaOH, the solution. Aliquots of incubation media and the solubilized tissue were pipetted into a scintillation vial containing Aquasol (New England Nuclear) and radioactivity was determined using a liquid scintillation counter (Packard Tricard 300C). TEA uptake by renal slices was expressed as the slice to medium (S/M) ratio; the concentration of the compund in the tissue (mole/g wet tissue) divided by that in the medium (mole/ml medium).

Measurement of oxygen consumption

The oxygen consumption of renal slices was measured with an oxygen monitor (Yellow springs Instrument Co., model 53). Approximately 50 mg of slices were incubated in a reaction vessel containing 4 ml of the modified Cross-Taggart medium saturated with oxygen

at 25°C. Decrease in PO_2 in the medium was measured using & Clark electrode for 15 min, and the rate of oxygen consumption was calculated.

Statistical analysis

The data are expressed as mean±SE and evaluated for significance using Student's t-test. A probability level of 0.05 was used to establish significance.

RESULTS

In vivo experiments

Table 1 summarizes the results of the clearance experiments. Ischemia for 30 min resulted in a marked increase in urine flow which was accompanied by an increase in urinary excretion of Na^+, K^+ and Cl^-. Similar changes in renal function were produced by 60 min of ischemia, but the effects were more pronounced. Such alterations in renal function did not return to the respective control values even when the kidney was allowed to be reciculated the blood for 2 h.

Slice experiments

Fig. 1 shows the effect of various durations of renal ischemia on TEA uptake. Accumulation of TEA in cortical slices obtained from kidneys occluded for 30 or 60 min was significantly increased as compared with that observed in the respective control. By 90 min of occlusion, however, TEA uptake was markedly depressed by 44% of the control.

In the next series of experiments, we examined the effect of reflow on alterations of TEA accumulation in renal cortex subsequent to ischemia. Figs. 2 and 3 depict the effects of reflow subsequent to 30 or 60 min of ischemia, respectively. When kidneys were allowed to reflow for longer than 30 min after 30 or 60 min of ischemia, TEA uptake in the ischemic

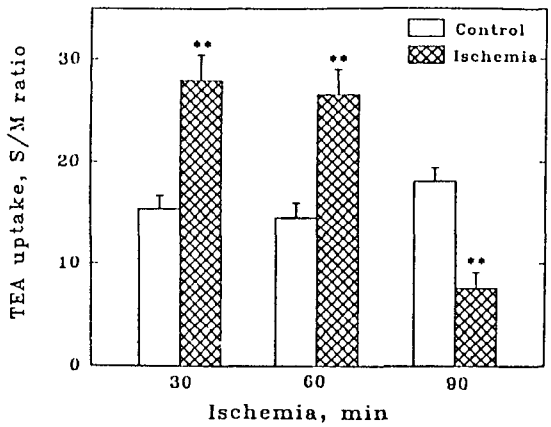

*Fig. 1. Alterations in accumulation of TEA in renal cortical slices obtained immediately after 30-to 90-min ischemia. The slices were incubated for 60 min at 25°C in a modified Cross-Taggart medium. The data are expressed as the mean ±SE of six experiments. **p<0.01 compared with the respective control.*

Table 1. Effect of ischemia on renal function

Ischemic Time (min)		Urine flow (μl/min/Kg)	$U_{Na}V$ (μEq/min/Kg)	U_kV (μEq/min/Kg)	$U_{Cl}V$ (μEq/min/Kg)
30	C	32±16	3.23±1.01	1.07±0.19	2.24±0.8
	I	73±15**	10.45±2.27**	2.69±0.53*	11.22±2.0**
60	C	25±12	2.14±1.11	1.01±0.15	1.47±0.28
	I	123±25**	14.43±3.10**	1.62±0.27*	11.35±2.32**

C, control(right) kideny; I, ischemia(left) kideny. All values were obtained during 2 h of reflow after ischemia. Data are mean±SE of ten animals.

*p<0.05; **p<0.01 compared with the respective control.

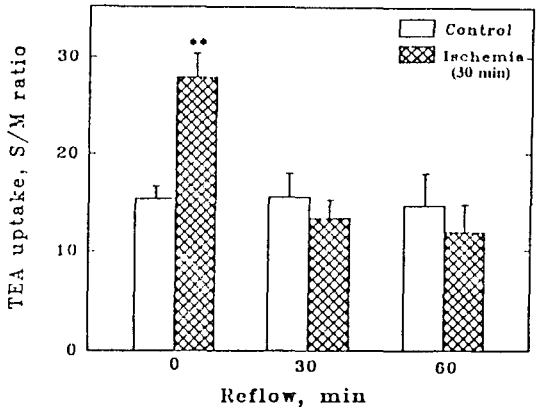

*Fig. 2. Effect of reflow after a 30-min ischemia on accumulation of TEA in renal cortical slices. The slices were incubated for 60 min at 25°C in a modified Cross-Taggart medium. The data are expressed as the mean±SE of four experiments. **p<0.01 compared with the control.*

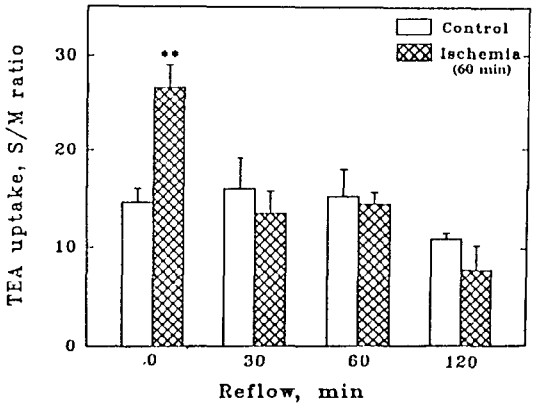

*Fig. 3. Effect of reflow after a 60-min ischemia on accumulation of TEA in renal cortical slices. The slices were incubated for 60 min at 25°C in a modified Cross-Taggart medium. The data are expressed as the mean±SE of four experiments. **p<0.01 compared with the control.*

kidney was not significantly different from that of the control kidney. With a 90 min ischemia, however, TEA uptake remained depressed even after 120 min of reflow (Fig. 4).

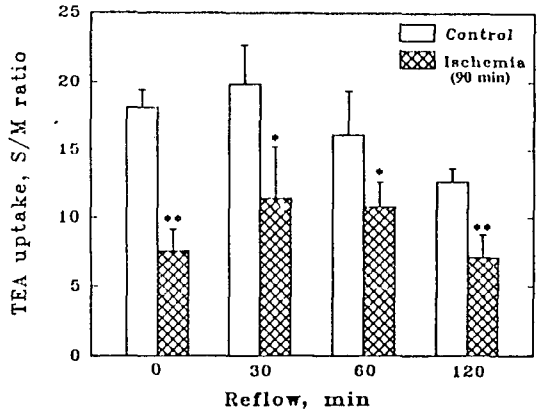

Fig. 4. Effect of reflow after a 90-min ischemia on accumulation of TEA in renal cortical slices. The slices were incubated for 60 min at 25°C in a modified Cross-Taggart medium. The data are expressed as the mean±SE of three experiments. **p<0.05; **p<0.01 compared with the respective control.

Table 2. Effect of ischemia on oxygen consumption in renal cortical slices

Ischemia (min)	Reflow (min)	O_2 consumption, μl/mg/min	
		Control	Ischemia
30	0	29.33±2.60	37.46±3.80*
	30	30.22±2.68	34.77±3.36
	60	34.89±2.17	36.66±3.55
60	0	19.31±2.23	20.36±2.22
	30	31.43±3.75	29.72±2.71
	60	41.34±3,39	38.66±2.73
	90	40.58±1.53	42.89±1.11
90	0	31.84±4.08	20.50±2.69*
	30	39.52±0.35	28.73±4.30*
	60	38.63±2.86	43.68±2.06
	90	29.74±2.90	24.24±2.26

Oxygen consumption was measured in a medium containing 130 mM NaCl, 10 mM KCl, 1.5 mM $CaCl_2$, 5 mM Na acetate and 20 mM Tris/HCl(pH 7.8). Values are the mean±SE of five experiments. *p<0.05 compared with the respecitve control.

In order to detrmine whether alterations in TEA uptake in ischemic kidneys were due to changes in energy metabolism, oxygen consumption of renal cortical slices was measured. The results are summarized in Table 2. The tissue oxygen consumption was significantly stimulated after 30 min of ischemia, which returned to the control level after 30 min of reflow. After 60 min of ischemia, however, oxygen consumption was not altered. When kidneys were made ischemic for 90 min oxygen consumption was significantly depressed, which was recovered to the control level after 60 min of reflow.

DISCUSSION

The present study demonstrates that TEA uptake in cortical slices is markedly stimulated when kidneys were exposed to ischemia for 30 and 60 min, whereas it is depressed after 90 min of ischemia. These results are inconsistent with studies by Berndt (1979) who found that occlusion of 45 min did not interfere with the accumulation of TEA in cortical slices. These results are also different from those of cleance experiments which demonstrated a decrease in TEA excretion of rat kidney (Shim, 1983; Lin & Lin, 1988). The reason for this discrepancy is not clear at present. However, similar results have been demonstrated in studies with nephrotoxic agents. Potassium dichromate, at different doses, was found to stimulate the organic cation system in rat renal cortical slices (Hirsch, 1973). In contrast, gentamicin reduced or did not alter N'-methylnicotinamide (NMN) uptake in rat renal cortical slices (Kluwe & Hook, 1978; Cohen et al, 1973; Hirsch, 1974).

Many organic cations are actively secreted by renal proximal tubule of the mammalian kidney (Rennick, 1981; Rennick & Farah, 1956). This secretion requires uptake from blood into the cell across the basolateral membrane of the proximal tubule epithelium and subsequent exit into the urine across the brush kidney cortex have consistently identified that organic cations are actively transported by a H^+/organic cation exchange process across the brush border membrane (Jung at al, 1989; Holohan & Ross, 1981; Takano et al, 1984; Wright et al, 1985). Transport of organic cation across the basolateral membrane has also been demonstrated to be an active process in studies with intact proximal tubule (Schëli et al, 1983; Tarloff & Brand, 1986), although studies with isolated basolateral membrane vesicles showed a facilitated diffusion process (Ross & Holohan, 1983). In tissue slices, the lumens of proximal tubules are collapsed which minimizes entry of TEA into the lumen (Burg & Orloff, 1969). Thus, the increased accumulation of TEA in cortical slices of ischemic kidneys indicates that the mechanism of TEA transport in the basolateral membrane was enhanced.

In this study, when renal blood flow was restored for as short as 30 min after 30 and 60 min of ischemia, TEA uptake by ischemic kidneys was returned to the control level. These findings suggest that the basolateral transport function can recover by 30 min of reflow following 60 min ischemia. Such results are of considerable interest since clamping of the renal artery for 30 and 60 min resulted in a polyuria which was accompanied by a marked increase in excretion of Na^+ during 2 h of reflow (Table 1), suggesting a significant impairment of tubular reabsorptive function. On the basis of these observations, we have assumed that the basolateral transport mechanisms are less susceptible to renal ischemia than those of luminal membrane.

Since the transport of TEA across the basolateral membrane of the renal proximal tubule is dependent on cellular metabolism (Nechay & Pardee, 1965; Rennick & Farah, 1956), it would be affected by an increase in the cellular metabolism. Keeler (1968) found that Na^+ increases and K^+ decreases in the proximal tubular cells after ischemia by the probable increased membrane permeability

in the presence of an intact Na^+-pump mechanism. In that case, a compensatory increase in Na^+-pump could cause a coupled increase in cell metabolism, thereby producing an increase in TEA uptake. In this study, the tissue oxygen consumption was significantly stimulated by a 30-min ischemia. After 60 min of ischemia, however, the oxygen consumption was not altered despite an increased TEA uptake. Thus, it seems unlikely that the alteration of metabolism is totally responsible for the increase in TEA uptake by ischemic kidney, although the increase in TEA uptake after 30 min of ischemia may be due to, in part, the stimulation of cellular metabolism. These findings on tissue oxygen consumption was in agreement with studies by other workers (Berndt, 1976; Randall, 1969; Reimer & Jennings, 1971). These results suggest that mitochondrial function in proximal tubules remains intact after periods of ischemia of 60 min despite evidence that the urine concentrating ability is significantly impaired.

The prolongation of ischemia by 90 min, in this study, caused a significant decrease in TEA uptake and tissue oxygen caused a significant decrease in TEA uptake and tissue oxygen consumption. The depressed uptake of TEA did not return to the control value even when kidneys were reperfused for 2 h after ischemia. However, the depressed oxygen consumption returned to the control level after 60 min of reflow. These results indicate that mitochondrial function is more resistant to ischemic injury than transport systems of membrane. These data tend to confirm in part earlier studies by Berndt (1976) who found that tissue oxygen consumption was not altered even after 90 min of occlusion, a time when electrolyte and the transport of organic compounds was seriously disrupted.

The present study shows clearly that TEA uptake was markedly stimulated in cortical slices of kidneys which had been ischemic for 30 and 60 min in vivo, duration at which tubular reabsorptive capacity of Na^+ and water was seriously impaired. The mechanisms underlying the augmented TEA uptake by cortical slices of ischemic kidneys remain to be explored. However, a similar phenomenon has been demonstrated in studies with potassium dichromate by Hirsch (1973). Thus, it seems likely that stimulation of organic cation transport system of proximal tubule by ischemia is not simply a specific response to ischemic injury.

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REFERENCES

Barnes JL, Osgood RW, Reineck HJ & Stein JH (1981) Glomerular alterations in an ischemic model of acute renal failure. *Lab Invest* **45**, 378-386

Berndt WO (1976) Effects of acute anoxia in renal transport processes. *J Toxicol Environm Health* **2**, 1-11

Burg MB & Orloff J (1969) p-aminohippurate uptake and exchange by separated renal tubules. *Am J Physiol* **217**, 1064-1068

Cohen L, Lapkin R & Kaloyanides GJ (1973) Effect of gentamicin on renal function in the rat. *J Pharmacol Exp Ther* **193**, 264-273

Donohoe JF, Venkatachalam AM, Bernard DB & Levinsky (1978) Tubular leakage and obstruction after renal ischemia: Structural-functional correlations. *Kid Int* **13**, 208-222

Finn WF (1981) Nephron heterogeneity in polyuric acute renal failure. *L lab Clin Med* **98**, 21-29

Herminghuysen D, Welbourne CJ & Welbourne TC (1985) Renal sodium reabsorption, oxygen consumption, and γ-glutamyltransferase excretion in the postischemic rat kidney. *Am J Physiol* **248**, F804-F809

Hirsch GH (1973) Differential effects of nephrotoxic agents on renal organic ion transport and metabolism. *J Pharmacol Exp Ther* **186**, 593-599

Hirsch GH (1974) Enhancement of gentamicin nephrotoxicity glycerol. *Toxicol Appl Pharmacol* **29**, 270-276

Holohan PD & Ross CR (1981) Mechanisms of organic cation transport in kidney plasma membrane vesicles. II. *pH studies. *J Pharmacol Exp Ther* **216**, 294-298

Johnston PA, Rennke H & Levinsky NG (1984) Recovery of proximal tubular function from ischemic injury. *Am J Physiol* **246**, F159-F166

Jung JS, Kim YK & Lee SH (1989) Characteristics of tetraethylammonium transport in rabbit renal plasma-membrane vesicles. *Biochem J* **259**, 377-383

Keeler R (1968) Composition and function of rat kidneys after transient nortmothermic ischemia. *Can J Physiol Pharmacol* **46**, 739-743

Kim YK, Lee SH, Goldinger JM & Hong SK (1986) Effect of ethanol on organic ion transport in rabbit kidney. *Toxicol Appl Pharmacol* **86**, 411-420

Kim YK, Park YS & Lee SH (1988) Effect of harmaline on organic ion transport in rabbit renal cortical slices. *Arch Int Pharmacodyn Ther* **294**, 259-272

Kluwe WM & Hook JB (1978) Functional nephrotoxicity of gentamicin in the rat. *Toxicol Appl Pharmacol* **45**, 163-175

Lin JH & Lin T-H (1988) Renal handling of drugs in renal failure I: differential effects of uranyl nitrate-and glycerol-induced acute renal failure on renal excretion of TEAB and PAH in rats. *J Pharmacol Exp Ther* **246**, 896-901

Medias NE, Donohoe JF & Harrington JT (1988) Postischemic acute renal failure. In: Brenner BM & Lazarus JM (ed) *Acute renal failure.* Chapter 9. Churchill Livingstone, New York, p 251-278

Molitoris BA & Kinne R (1987) Ischemia induces surface membrane dysfunction mechamism of altered Na^+-dependent glucose transport. *J Clin Invest* **80**, 647-654

Myers BD, Miller DC, Mehigan JT, Olcott C, Golbetz H, Robertson CR, Dertby G, Spencer R & Friedman S (1984) Nature of the renal injury following total renal ischemia in man. *J Clin Invest* **73**, 329-341

Nechay BR & Pardee LM (1965) Inhibition of N'-methylnicotinamide secretion by ouabain in the chicken kidney. *J Pharmacol Exp Ther* **147**, 270-276

Randall HM, Jr (1969) Effects of acute renal ischemia on aerobic metabolism of dog kidney homogenates. *Am J Physiol* **217**, 1413-1418

Reimer KA & Jennings RB (1971) Alterations in renal cortex following ischemic injury. I. PAH uptake by slices of cortex after ischemia or autolysis. *J Clin Invest* **25**, 176-184

Rennick BR (1981) Renal tubule transport of organic cations. *Am J Physiol* **240**, F83-F89

Rennick BR & Farah A (1956) Studies on the renal tubule transport of tetraethylammonium ion in the dog. *J Pharmacol Exp Ther* **116**, 287-295

Ross CR & Holohan PD (1983) Transport of organic anions and cations in isolated renal plasma membranes. *Ann Rev Pharmacol Toxicol* **23**, 65-85

Schàli C, Schild L, Overney J & Roch-Ramel F (1983) Secretion of tetraethylammonium by proximal tubules of rabbit kidneys. *Am J Physiol* **254**, F238-F246

Shim CK, Dawada Y, Oga T & Hanano M(1983) Effect of experimental acute renal failure on intrinsic renal tubular secretory clearance of organic cations in rats. *J Pharm Dyn* **6**, 787-789

Stein JH, Lifschitz MD & Barnes LD(1978) Current concepts on the pathophysiology of acute renal failure. *Am J Physiol* **234**, F172-F181

Takano M, Inui K-I, Okano T, Saito H & Hori R (1984) Carrier-mediated transport systems of teraethylammonium in rat renal brush border and basolateral memnbrane vesicles. *Biochim Biophys Acta* **773**, 113-124

Tarloff JB & Brand PH(1986) Active tetraethylammonium uptake across the basolateral membrane of rabbit proximal tubule. *Am J Physiol* **251**, F141- F149

Wright SH(1985) Transport of N'- methylnicotinamide across brush border membrane vesicles from rabbit kidney. *Am J Physiol* **249**, *F903-F911*