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Protective effect of prostacyclin on postischemic acute renal failure in the rat

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Protective effect of prostacyclin on postischemic acute renal failure in the rat. Infusion of prostacyclin (PGI_2) reportedly attenuates renal ischemic injury in the dog and the rat. In the dog, PGI_2 is a potent renal vasodilator; in the rat a direct action on the renal vasculature is not always apparent. To determine whether or not the protective effect of PGI_2 on postichemic ARF was hemodynamically mediated, studies were performed in uninephrectomized Sprague–Dawley rats before and after a 40 minute period of complete renal artery occlusion. In response to the preischemic infusion of PGI_2 for 30 minutes at 160 ng/kg body wt/min i.v. ($N = 7$), MAP and RBF fell to 86 ± 7% ($P < 0.0001$) and 84 ± 9% ($P < 0.05$) of baseline values, respectively. RVR initially declined to 81 ± 9% of baseline values ($P < 0.025$) but returned to 102 ± 13% of baseline values prior to the period of ischemia. Following the period of ischemia, reflow of blood in the rats receiving PGI_2 was delayed when compared to rats not receiving PGI_2 ($N = 7$). RBF returned to only 76 ± 19% of the initial values in PGI_2-treated rats ($P < 0.01$) but to 90 ± 12% of the initial values in rats receiving buffer alone (NS). Observations made during the ensuing 48 hours in animals treated with either 80 ($N = 8$) or 160 ng/kg/body wt/min ($N = 7$) for 30 minutes before and four hours after the period of ischemia indicated that renal function improved to a greater extent in the PGI_2-treated animals than in buffer–treated animals ($N = 15$) as judged by significantly–greater mean values of V, U_{Osm}, U_{Cr} and C_{Cr}. On the second day after ischemia, C_{In} was significantly greater in PGI_2-treated animals than in the postischemic animals receiving buffer alone (77 ± 45 vs. 33 ± 20 μl/min/100 g body wt; $P < 0.05$) despite the fact that no differences were found in the mean values of RBF (3.59 ± 1.08 vs. 3.43 ± 0.32 ml/min/100 body wt. Blinded analysis of the histological sections revealed significantly less evidence of tubular epithelial cell necrosis in the PGI_2-treated animals ($P < 0.005$). The data indicate that the protective effect of PGI_2 on the renal response to ischemic injury in the Sprague–Dawley rat is not related to changes in RBF or RVR. Instead, the beneficial effect of PGI_2 may be a result of cytoprotective properties as has been demonstrated in other tissues.

In addition to its ability to prevent platelet aggregation, prostacyclin (PGI_2, epoprostenol) is a potent systemic vasodilator and may have cytoprotective properties. Although several studies have demonstrated that the severity of postischemic acute renal failure in both the dog and rat may be decreased by treatment with PGI_2 [1–6], the mechanism by which this occurs has not been established. Infusion of PGI_2 results in direct renal vasodilation in the dog [7–9]; it has been suggested that this effect is responsible for the improvement in renal function following ischemic injury. In the rat [10–14], however, it has been difficult to demonstrate that infusion of PGI_2 results in a significant increase in renal blood flow (RBF) [15]. In fact, an increase in renal vascular resistance (RVR) has been noted in response to an intra-aortic infusion of 62 ng/kg/min in rats [16]. At intravenously–administered doses greater than 40 to 80 ng/kg/min, systemic vasodilation and a fall in arterial blood pressure (MAP) are accompanied by a decrease in RBF [17]. These considerations raise the possibility that the protective effect of PGI_2 on renal ischemic injury is not a consequence of an increase in RBF.

To examine the effect of high doses on PGI_2 on RBF following ischemic injury to the kidney and to determine if an increase in RBF or a decrease in RVR was necessary for the protective effect to be manifested, a series of studies was performed before and during the 48 hours following a 40-minute period of renal ischemia. The results indicate that the beneficial effect of PGI_2 is not a result of an increase in the rate and/or extent of reperfusion of the postischemic kidney and suggest that other mechanisms are responsible.

Methods

Observations are reported on 50 male Sprague–Dawley rats that weighed between 275 and 325 gms each. In the first series of experiments, all animals ($N=14$) were fasted overnight but allowed free access to water. On the day of surgery the animals were anesthetized with intraperitoneal sodium pentobarbital, 60 mg/kg/ body weight, and placed on a heated table that maintained body temperature between 37 and 38°C. A tracheostomy was performed, and a catheter was placed in a jugular vein for the infusion of 0.85% sodium chloride (at a rate of 40 μl/min) and the administration of supplemental doses of sodium pentobarbital. The right kidney was exposed through a flank incision, the pedicle ligated, and the kidney removed. After the incision was closed, the left kidney was exposed through an abdominal incision and the left renal artery was isolated. RBF was measured with a non-cannulating electromagnetic flow transducer connected to a square–wave flowmeter and a Hewlett–Packard recorder (Elkhart, Indiana, USA) [18]. Femoral MAP was recorded continuously with a Statham P23Db pressure transducer (Statham Instruments, Oxnard, California, USA) connected to a Hewlett–Packard recorder.

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After the surgery was complete, MAP and RBF were measured for 10 min (Period 1). Thereafter, the animals were divided into two groups. In the treated group (N = 7), PGI_2 (Burroughs Wellcome Co., Research Triangle Park, North Carolina, USA) was dissolved in glycine buffer and infused through the catheter in the jugular vein at 160 ng/kg/min (Period 2). After 30 minutes, 10 to 15 units of heparin/100 g body weight (1000 units/ml, the Upjohn Company, Inc., Kalamazoo, Michigan, USA) were injected intravenously, and a smooth-surfaced tension clamp (Schwartz 1-inch clip; Roboz Surgical Instrument Co., Inc.) was positioned on the left renal artery so that it was completely occluded. The clamp was removed after 40 minutes. PGI_2 was infused continuously during this time and for an additional 30 minutes after the release of the clamp (Period 3). Then, the PGI_2 was stopped, and MAP and RBF were monitored for a final 10 minutes (Period 4). At each interval RVR was calculated according to the equation:

$$RVR = MAP - MVP/RBF$$

where MVP is the mean renal venous pressure and is assumed to be 3 mm Hg. The second group of rats (N = 7) was treated similarly, but was infused with the glycine buffer alone.

In the second series of experiments, rats (N = 36) were anesthetized by intraperitoneal injection of sodium pentobarbital and placed on a heated table A Silastic catheter was placed in a jugular vein and tunneled subcutaneously to an incision in the dorsal surface of the neck. The catheter was passed through a stainless steel spring that was sutured in place. The spring was attached to a brass swivel, which was attached by a second catheter to a constant infusion pump. The right kidney was removed through a flank incision as described above. In the majority of rats (N = 30) the left renal artery was exposed by a small midline abdominal incision and it was isolated and completely occluded for 40 minutes as described above. After removal of the clamp, the surface of the kidney was observed and the time of blood reflow — as judged by diffuse capillary filling — was recorded. In the remaining rats (N = 6) the left renal artery was exposed and isolated but was not clamped. Thereafter, the wound was closed, and the animals were placed in individual metabolic cages. Saline was then infused at approximately 1 ml/hr for the duration of the experiment. The animals were not restrained; connection to the brass swivel allowed free movement in the cages.

In one group of animals, PGI_2 at either 80 (N = 7) or 160 (N = 8) ng/kg/min was infused for 30 minutes before, during and for four hours after the period of renal ischemia. Four hours was selected as the duration of the postischemic infusion of PGI_2 to include the time over which reperfusion injury occurs. The second group of animals (N = 15) was treated similarly but received glycine buffer alone. The animals not subjected to renal ischemia also received glycine buffer alone. All animals were maintained on their standard rat pellet diet (Purina) and allowed free access to water. Urine was collected continuously during two 24-hour periods. Blood samples were obtained at the end of each period from the tip of the tail. The hematocrit was measured in heparinized capillary tubes. Plasma and urine creatinine concentrations (P_{Cr}, and U_{Cr}) were determined by a modification of the method of Chasson, Grady and Stanley [19]. Creatinine clearance (C_{Cr}) was calculated for each 24-hour

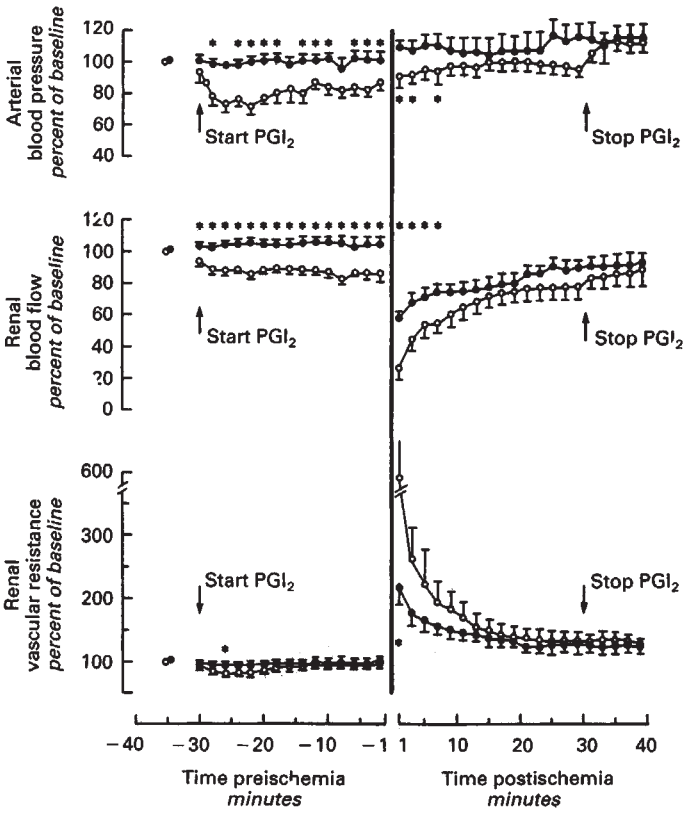

Fig. 1. *The effects of an infusion of PGI_2 (160 ng/kg/min) or glycine buffer alone on arterial blood pressure, renal blood flow and renal vascular resistance before and after a 40-minute period of complete renal artery occlusion in uninephrectomized rats.* Results are illustrated as percent of baseline values ± SEM. Symbols are: (●) control, (○) PGI_2-treated, $^*P < 0.05$.

pressure method using a Wescor osmometer and osmolar excretion ($U_{Osm}V$) was calculated. Plasma and urine sodium concentrations (P_{Na}, U_{Na}) were determined by flame photometry (model 480, Corning Medical and Scientific, Midfield, Massachusetts, USA), and the fractional excretion of sodium (FE_{Na}) was calculated.

At the end of 48 hours, the animals were anesthetized by intravenous injection of sodium pentobarbital and prepared for clearance studies. A tracheostomy was performed, and a femoral artery was cannulated for determination of MAP. The left kidney was exposed through an abdominal incision. The left ureter was catheterized with PE-10 polyethylene tubing. RBF was determined as described above. After the surgical procedure was completed, an appropriate amount of ^{3}H-methoxy inulin (New England Nuclear, Boston, Massachusetts, USA) was added to the saline infusion. After equilibration for 60 minutes urine was collected in preweighed cups during two 15-minute periods. Midway through each period, a blood sample was obtained from the tip of the tail for determination of hematocrit and inulin concentration. Urine and plasma ^{3}H activities were measured in a liquid scintillation counter (Packard Instrument Company, Downers Grove, New Jersey, USA).

Table 1. Effect of infusion of PGI_2 (160 ng/kg/min; $N = 7$) or Buffer alone ($N = 7$) on arterial blood pressure, renal blood flow and renal vascular resistance before and after a 40-minute period of complete renal artery occlusion in uninephrectomized rats

	Preischemia		Postischemia	
	Period 1	Period 2	Period 3	Period 4
ABP *mm hg*				
Buffer	112 ± 10	109 ± 16	125 ± 23	124 ± 21
PGI_2	110 ± 13	90 ± 13	100 ± 13	115 ± 6
P	NS	<0.025	<0.025	NS
RBF *ml/min per 100 g body wt*				
Buffer	3.82 ± 0.43	3.87 ± 0.38	3.21 ± 0.39	3.36 ± 0.68
PGI_2	3.82 ± 0.92	3.13 ± 0.38	2.86 ± 0.74	3.18 ± 0.62
P	NS	<0.0125	NS	NS
RVR *mm Hg/ml/min per 100 g body wt*				
Buffer	28.33 ± 4.03	27.65 ± 55.86	38.66 ± 3.11	36.28 ± 5.97
PGI_2	29.10 ± 5.27	28.66 ± 3.16	36.03 ± 5.23	36.05 ± 5.23
P	NS	NS	NS	NS

Table 2. Effects of PGI_2 infusion (80 and 160 ng/kg/min) 30 minutes before and four hours after a 40-minute period of complete renal artery occlusion in uninephrectomized rats

			Postischemic		
	Day	Non-ischemic	Buffer	*P*	PGI_2
Hematocrit %	1	41.3 ± 3.8	37.5 ± 3.7	<0.01	40.8 ± 2.9
	2	40.8 ± 3.7	37.8 ± 4.09	<0.0025	42.5 ± 3.3
Serum creatinine *mg/dl*	1	0.3 ± 0.1	4.1 ± 0.6	NS	3.7 ± 0.6
	2	0.3 ± 0.1	5.1 ± 1.0	<0.025	3.7 ± 1.2
Urine volume *ml/day/100 g body wt*	1	9.5 ± 1.5	8.3 ± 4.1	<0.05	11.0 ± 4.3
	2	7.7 ± 2.6	13.0 ± 5.6	<0.0025	18.9 ± 4.5
Water intake *ml/day/100 g body wt*	1	3.9 ± 3.7	6.7 ± 2.4	<0.005	10.5 ± 4.9
	2	6.5 ± 3.0	7.4 ± 3.9	<0.005	11.8 ± 4.8
Saline infusion *ml/day/100 g body wt*	1	7.2 ± 1.1	6.8 ± 1.0	NS	7.6 ± 1.0
	2	5.7 ± 1.7	6.4 ± 0.9	NS	7.0 ± 1.1
Net in *ml/day/100 g body wt*	1	1.4 ± 5.2	5.1 ± 2.9	NS	7.1 ± 5.9
	2	4.5 ± 3.3	0.8 ± 3.9	NS	−0.1 ± 3.2
Urine osmolality *mOsm/liter*	1	814 ± 333	482 ± 117	<0.0005	641 ± 75
	2	1140 ± 374	464 ± 125	<0.0005	653 ± 101
Osmolar excretion *mOsm/day/100 g body wt*	1	7.4 ± 2.3	4.1 ± 2.5	<0.0025	7.0 ± 2.7
	2	8.3 ± 2.3	6.4 ± 4.1	<0.0005	12.2 ± 3.0
Urine creatinine *mg/dl*	1	30.1 ± 10.3	7.2 ± 2.6	NS	9.2 ± 3.9
	2	33.9 ± 16.7	9.1 ± 3.9	<0.05	12.3 ± 5.7
Creatinine excretion *μg/min/100 g body wt*	1	20.0 ± 4.6	4.1 ± 2.8	<0.025	6.6 ± 2.9
	2	17.5 ± 6.8	8.2 ± 4.4	<0.0005	15.1 ± 4.9
U/P creat ratio	1	101 ± 50	1.8 ± 0.8	NS	2.6 ± 1.3
	2	162 ± 94	1.9 ± 1.1	<0.0125	4.0 ± 2.5
Creatinine clearance *μl/min/100 g body wt*	1	667 ± 309	11 ± 8	<0.05	18 ± 12
	2	830 ± 466	18 ± 12	<0.0025	46 ± 28

Results are ±1 SD. Reference values obtained from uninephrectomized rats not subjected to ischemic injury also listed.

injury were excised, cleared of perirenal fat, decapsulated, and fixed in formalin. Kidneys from rats treated with PGI_2 or the glycine buffer alone were coded and prepared for light microscopy. Following a blinded histopathologic analysis, the kidneys were assigned an injury score based on the severity and extent of the tubular epithelial cell injury. The degree of necrosis was rated as minimal, mild, moderate, or marked and assigned a value form 1 to 4.

For analyses of significance, paired and unpaired *t*=tests and linear regression by least-squares were performed for the functional studies. Differences in the histological studies were evaluated with the Wilcoxon non-paired rank sum test. A *P* value greater than 0.05 was considered to be not statistically significant (NS). Values are presented as means ± SD unless otherwise indicated.

Results

Absolute mean values of MAP, RBF, and RVR observed in glycine buffer and PGI_2-treated animals at the end of the two preischemic and the two postischemic periods are listed in Table 1. The mean values, expressed as percent of baseline, are illustrated in Figure 1. Before the infusion of PGI_2 or glycine buffer alone (Period 1), there were no significant differences in the baseline values of MAP, RBF, or RVR. In response to the PGI_2 infusion (Period 2), MAP fell to 73 ± 12% of baseline at 5 minutes ($P < 0.001$) and then returned to 86 ± 7% of baseline prior to the period of ischemia ($P < 0.001$). In association with the decline in MAP, RBF fell to 88 ± 7% of baseline at 5 minutes ($P < 0.025$) and did not change thereafter despite the increase in MAP. Before the period of ischemia, RBF was 84 ±

Table 3. Results of clearance studies in untreated and PGI_2-treated, unilaterally nephrectomized rats 48 hours following a 40-minute period of complete renal artery occlusion

		Postischemic		
	Nonischemic	Untreated	*P*	PGI_2
Arterial blood pressure *mm Hg*	121 ± 13	102 ± 18	<0.05	118 ± 10
Renal blood flow *ml/min/100 g body wt*	4.12 ± 0.79	3.43 ± 0.32	NS	3.59 ± 1.08
Renal vascular resistance *mm Hg/ml/min/100 g body wt*	29.64 ± 7.21	29.98 ± 7.75	NS	34.01 ± 8.19
Urine/plasma inulin ratio	123.4 ± 42.8	5.0 ± 1.4	NS	6.0 ± 3.2
Urine flow rate *ul/min/100 g body wt*	6.5 ± 4.1	6.9 ± 4.6	<0.05	12.9 ± 4.6
Insulin clearance *ul/min/100 g body wt*	653 ± 132	33 ± 20	<0.05	77 ± 45
N of animals	6	15		15

Results ±1 SD. Reference values obtained from uninephrectomized rats not subjected to ischemic injury also listed.

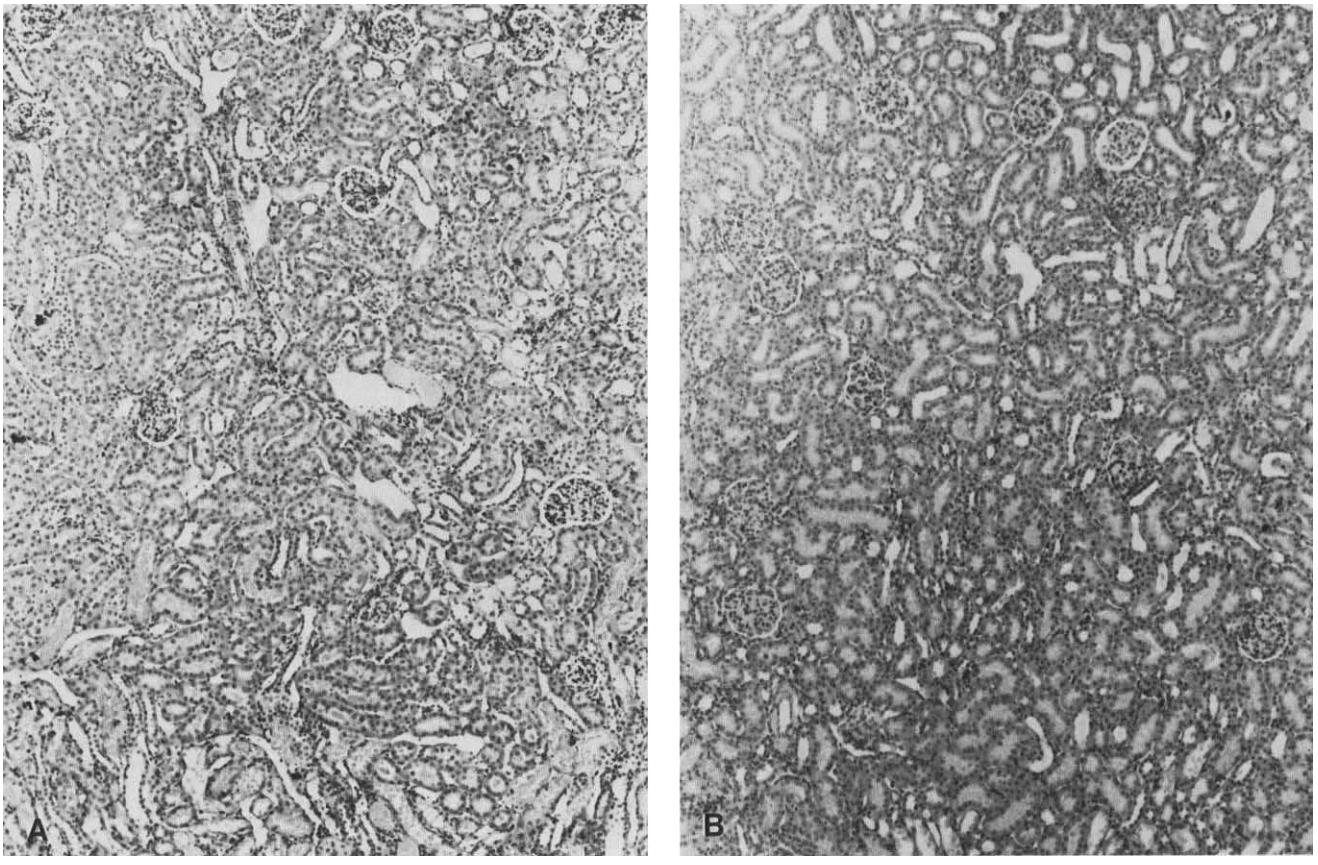

Fig. 2. *Light micrograph of representative sections demonstrating a) minimal (score = 1) and b) mild (score = 2) tubular necrosis from prostacyclin–treated animals 48 hours after ischemic injury.* Tubular dilatation, atrophy and casts are confined mainly to the inner cortex (hematoxylin and eosin, ×120).

9% of baseline ($P < 0.025$). RVR exhibited an initial fall to 81 ± 9% of baseline at 5 minutes ($P < 0.025$). This was followed by a gradual increase so that prior to the period of ischemia, RVR was 102 ± 13% of baseline (NS). These values remained constant in the animals receiving the glycine buffer alone.

Upon release of the renal artery clamp (Period 3), there were significant differences in the pattern of reflow (Fig. 1) that were most evident during the first 5 minutes. During this time, the increase in RBF in the PGI_2-treated animals was delayed when compared to the animals receiving the glycine buffer alone. In part, this was a result of a lower MAP in the PGI_2-treated animals and in part a result of a higher RVR.

Thirty minutes after the release of the renal artery clamp in the PGI_2-treated animals, RBF had only returned to 76 ± 19% ($P < 0.01$) of baseline; in the animals receiving the glycine buffer alone, RBF returned to 90 ± 12% of baseline (NS). When the PGI_2 infusion was stopped (Period 4), RBF increased to 89 ± 15% of baseline (NS). This value was not significantly

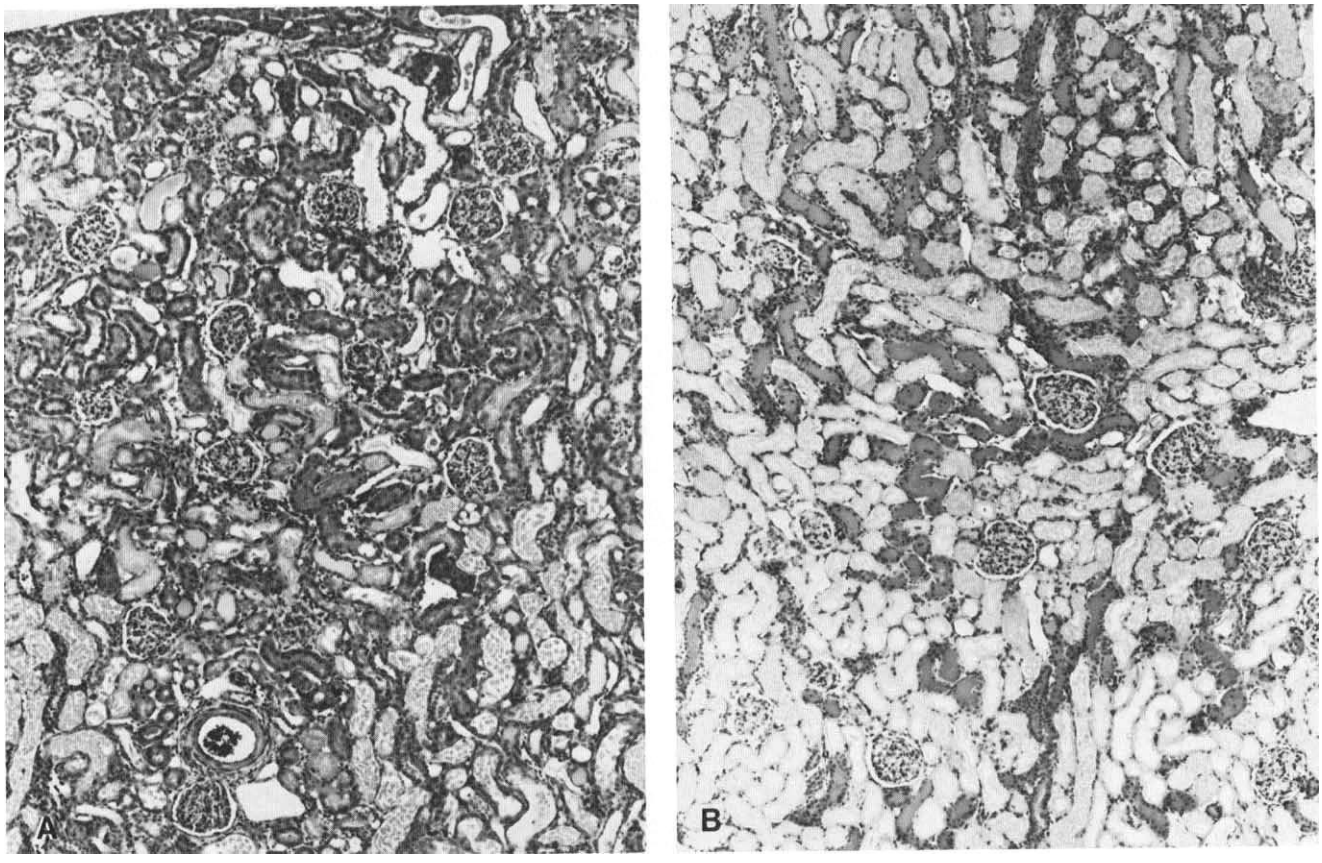

Fig. 3. *Light micrograph of representative sections demonstrating a) moderate (score = 3) and b) marked (score = 4) tubular necrosis from buffer–treated animals 48 hours after ischemic injury.* Tubular dilatation, atrophy and casts involve the full width of the cortex (hematoxylin and eosin, ×120).

different from that observed in the animals receiving the glycin buffer alone. The increase in RBF that occurred when the PGI_2 infusion was stopped resulted from an increase in MAP, as RVR remained constant.

The changes in renal function that occurred during the two days of observations are listed in Table 2. Also listed are the results obtained in unilaterally nephrectomized rats not subjected to a period of renal ischemia. No significant differences were found in the response to 80 or 160 ng/kg body weight/min, and the results are combined. Inspection of the kidney surface during the time of reflow demonstrated a delay in capillary filling of PGI_2-treated animals compared with animals receiving glycine buffer alone (3.4 ± 1.2 vs. 1.9 ± 0.9 min; $P < 0.0005$). This was consistent with the differences in the pattern of reflow noted above and illustrated in Figure 1. Over the 48-hour period of observation following renal ischemia, urine output (V) was 41% greater in PGI_2-treated animals than in the animals given glycine buffer alone. This was accompanied by a 59% greater intake of water, so the net difference between intake and output in the two groups was similar. Judged by differences in the hematocrit, there was no evidence of hemodilution in the PGI_2-treated animals. In fact, on both days, the hematocrit was significantly less in the animals receiving the glycine buffer alone.

In PGI_2-treated animals, the mean values of U_{Osm} on both the first and second postischemic day were significantly greater than those animals receiving the glycine buffer alone. The mean values of U_{Cr} in the two groups reflected the differences in U_{Osm} and were significantly greater on day 2 in the PGI_2-treated animals. As a result of the higher values of V, U_{Osm} and U_{Cr}, the mean values of $U_{Osm}V$ and $U_{Cr}V$ were significantly greater in the PGI_2-treated animals than in the animals receiving the glycine buffer alone on both the first and second postischemic days. Although a significant difference in the mean values of S_{Cr} was not present on day 1, the mean value of the C_{Cr} was 64% greater in the PGI_2-treated animals. A significant difference in the mean values of S_{Cr} was evident on day 2, and at this time the mean value of the C_{Cr} was 156% greater in the PGI_2-treated animals.

Results of the clearance studies on day 2 are listed in Table 3. The mean values for MAP, RBF, and RVR in glycine buffer and PGI_2-treated rats were similar with mean MAP slightly higher in the PGI_2 group. In the PGI_2-treated rats, the mean value of V and C_{In} were 87% and 148% greater than the respective values found in the animals receiving the glycine buffer alone. These differences were similar to the differences in V and C_{Cr} noted previously.

The histologic examination of all kidneys from the glycine buffer and PGI_2-treated animals showed evidence of ischemic

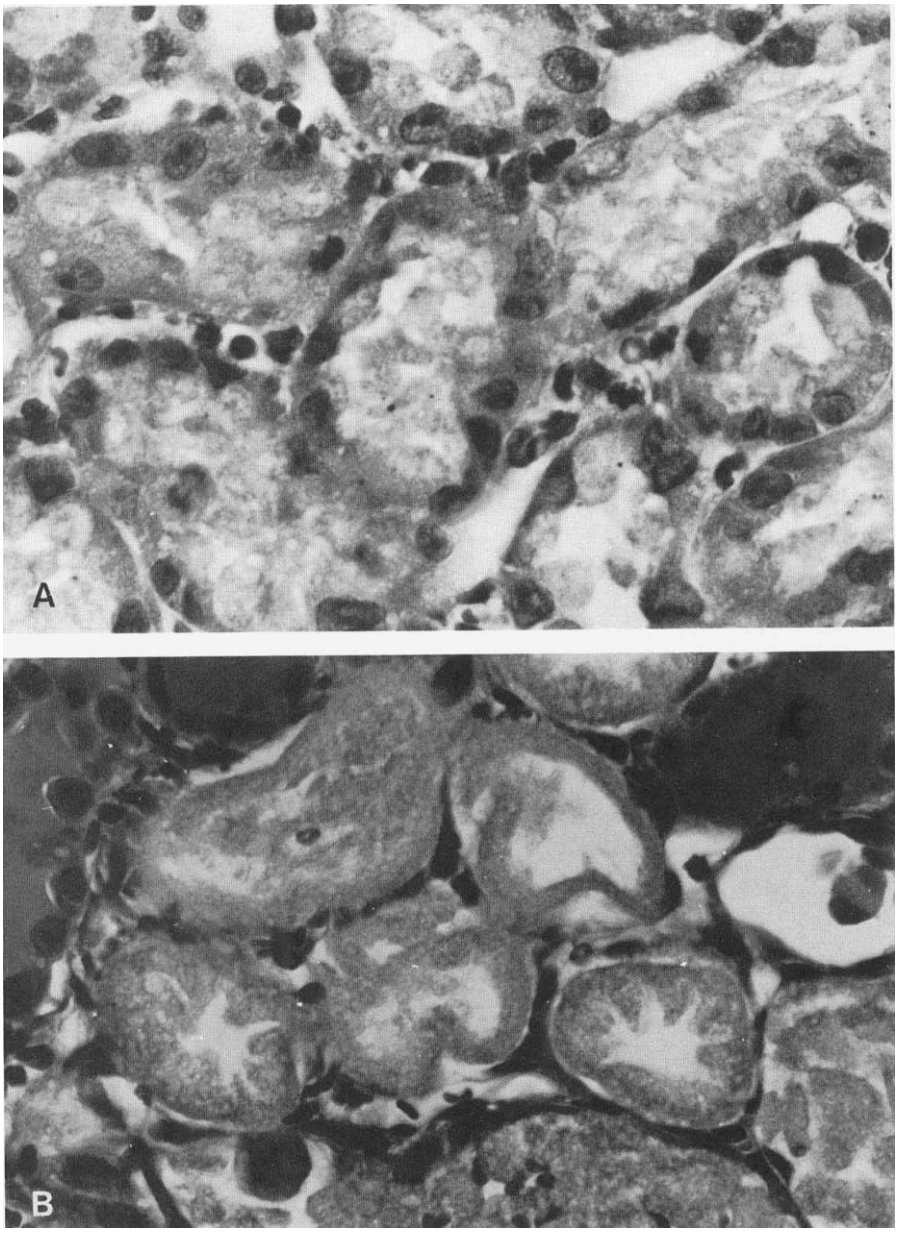

Fig. 4. *Light micrographs at higher magnification of sections from a) prostacyclin–treated and b) buffer–treated animals 48 hours after ischemic injury.*In rats given prostacyclin, tubular lumena are filled with proteinaceous material. Basement membranes are lined with cuboidal and rhomboidal shaped epithelial cells. Nuclei in the majority of the tubular epithelial cells are distinct and some contain mitotic figures. In rats given buffer alone, tubular epithelial cell damage is more severe. Tubular lumena contain remains of tubular epithelial cells while segments of the basement membrane are uncovered. In many areas necrosis of tubular epithelial cells has progressed to the point that cell boundaries and intracellular structures cannot be recognized. (hematoxylin and eosin, ×340).

injury and the presence of proteinaceous and granular cast formation. A minimal amount of intratubular mineralization with areas of tubular regeneration was observed equally in both groups. Based on the assigned severity scores, kidneys from the animals receiving glycine buffer alone exhibited significantly greater tubular epithelial cell necrosis than did the kidneys from the PGI_2-treated animals (3.27 ± 0.79 vs. 2.27 ± 0.59; $P < 0.005$). In addition, the injury involved the full width of the cortex in 81.8% (9 of 11) of the control kidneys and only 33.3% (5 of 15) of the PGI_3-treated kidneys. Representative photomicrographs are shown in Figures 2 through 4.

An inverse relationship was found between the severity of the tubular epithelial cell necrosis in individual animals and the corresponding C_{Cr} ($r = 0.643$; $P < 0.001$) while a positive correlation was found when the histologic score was compared to the corresponding FE_{Na} ($R = 0.513$; $P < 0.005$).

Discussion

These data indicate the protective effect of PGI_2 is not a result of renal vasodilation and an increase in RBF. Compared with animals receiving glycine buffer alone, those treated with PGI_2 had higher C_{Cr} and C_{In} 48 hours following ischemic injury in associations with greater V and less severe derangements in tubular function as indicated by mean values of U_{Osm} and U_{Cr}. Examination of histologic sections revealed that the degree of tubular epithelial cell injury could be correlated with reduction in C_{Cr} and was less severe in the animals treated with PGI_2. These observations are consistent with the hypothesis that the beneficial effect of PGI_2 is a result of a protective action on renal tubular epithelial cells. It should be emphasized that although C_{Cr} and C_{In} in the PGI_2 treated animals remained severely depressed when compared to the values obtained in animals not subjected to renal ischemia, the reduction in tubular epithelial cell injury was substantial. This supports the notion that relatively mild degrees of anatomical injury may be associated with severe functional impairment.

Previous studies concerning the effect of PGI_2 on postischemic ARF have been performed in both the dog and rat. In the dog, PGI_2 at doses of 0.25 μg/kg body weight/min administered prior to autotransplantation [1] or renal pedicle cross clamping [2] significantly minimized the extent of ischemic injury. In both studies, prior vasodilation was considered a contributor to the protective effect. In the rat, doses as high as 0.5 μg given directly into the renal artery [3, 4] and as low as 8 ng/kg body weight/min infused intravenously in both hydropenic and volume expanded animals [5] have had a protective effect. While these studies relate to the effect of exogenously–administered PGI_2, evidence also exists for a protective role of endogenous PGI_2. Observations made in the rat show that the beneficial effect of thromboxane synthetase inhibition on postischemic ARF is more closely related to the associated increase in PGI_2 synthesis rather than a direct result of the inhibition of thromboxane production [6].

It has been reported that a more rapid return of blood flow in the immediate postischemic period may be accompanied by an initial increase in glomerular filtration rate, urine flow, and solute excretion [2]. PGI_2 is a potent systemic vasodilator and, in the dog, is capable of directly reducing RVR [7–9]. In the rat, it has been suggested that PGI_2 also has a direct renal vasodilatory action [10–14] although not all agree [10, 16]. Confounding the issue are the observations that PGI_2 stimulates renin release in intact animals [7] and in preparations of isolated glomeruli [21]. Also, strain differences may exist in the renal vascular response to PGI_2. Notably, when Sprague–Dawley rats are pretreated with saralasin, the intra-aortic infusion of PGI_2 at a dose sufficient to produce significant systemic vasodilation (3.6 μg/kg/hr), does not alter RVR [15]. When PGI_2 is infused intravenously in rats at much larger doses (1 to 1265 ng/100 g body weight/min), a fall in MAP is accompanied by a decline in RVR without a change in RBF. The decrease in RVR appears to be a result of the autoregulatory response to a decrease in perfusion pressure rather than a direct action of PGI_2 [17].

In this study, the infusion of PGI_2 resulted in a fall of MAP and RBF. When the PGI_2 infusion was stopped, MAP and RBF rose to levels similar to those observed in animals not treated with PGI_2. During the initial postischemic period, the return of RBF was more rapid and of greater magnitude in control animals than in animals treated with PGI_2. The reason for the delay was not entirely due to a lower perfusion pressure in the PGI_2-treated animals in that RVR during the very early postischemic period was significantly greater in this group. It is possible that PGI_2-stimulated renin release contributed to these differences. When RBF was measured 48 hours after the insult, differences were not found between the two groups of animals. Thus, the beneficial effect of PGI_2 was not related to an increase in RBF in the immediate postischemic period, or to a greater rate of perfusion as recovery occurred.

PGI_2 is a potent inhibitor of platelet aggregation. It is possible that platelet aggregation accompanies renal ischemic injury and that the subsequent release of vasoactive substances contributes to the increase in RVR and poor perfusion of the postischemic kidney. In our study, PGI_2 was administered in doses sufficient to inhibit platelet function. As indicated above, this was not reflected in an improvement in RBF or RVR. A prominent feature of ischemic acute renal failure in the rat is the appearance of marked medullary hyperemia, which appears to be due to the accumulation of aggregated erythrocytes in inner stripe capillaries. The magnitude of these anatomical changes can be directly related to the severity of the functional abnormalities. The vascular congestion does not appear to be a result of fibrin deposition or platelet aggregation but rather has been related to the occlusion of vasa rectae by erythrocytes [22]. In this regard, it has been observed that a reduction in the hematocrit is associated with less severe injury following ischemic insults [23]. The hematocrit on both days 1 and 2 was significantly higher in the PGI_2-treated rats in our study.

A number of diverse studies have indicated that PGI_2 possesses cytoprotective properties. Lefer et al [24] were the first to note its beneficial effect in acute myocardial ischemia. Others have demonstrated that various prostaglandins exert a protective effect on the gastric mucosa [25, 26]. PGI_2 has also been shown to reduce the breakdown of membrane phosphatides [27], preserve the integrity of platelets during storage [28], protect hepatic cells against ischemic [29] and toxic [30] injury, and modify the changes of acute experimental pancreatitis in dogs [31]. It has been suggested that the mechanism by which PGI_2 exerts its cytoprotective action may be related to changes in membrane transport processes, increases in tissue levels of adenosine triphosphate and other cyclic nucleotides [29, 32], or alterations in the enzyme systems generating toxic–free radical anions [33]. The latter mechanism may involve a direct cellular action [34] or may be a result of the inhibition of oxygen centered radical formation from neutrophils [35–37]. Other undefined mechanisms of cell preservation may also be influenced by PGI_2 [38]. Alternatively, prostanoids may accelerate certain restorative metabolic and structural processes rather than minimize the role of free–radicals and lipoperoxides [30].

It has been demonstrated that the administration of specific free–radical scavengers [39] or treatment with calcium channel–blocking agents [40] reduces the amount of tubular epithelial cell injury associated with renal ischemia. Moreover, ATP-$MgCl_2$ solutions [41] have been shown to minimize ischemic damage and/or promote early recovery. The results of the present study support the concept that PGI_2 has cytoprotective properties that influence the response to ischemic injury. Recently, studies performed in the isolated perfused rat kidney

have indicated that the S1 and S2 segments of the proximal tubule, the S3 segment of the proximal tubule and the medullary thick ascending limb differ in their sensitivity to hypoxic injury; also, agents that influence the degree of injury in one segment may not be as effective in other segments [42, 43]. Although we did not examine the specific nephron segment at which PGI_2 may have exerted its protective effects, the observation that attenuation of the ischemic injury in the PGI_2-treated animals was most apparent in the outer cortex suggests PGI_2 may have had an effect on proximal tubular epithelial cells. However, the greater values of U_{Osm} and U_{Cr} are consistent with a more distal effect.

Finally, it is possible that other cytoprotective agents may interact with PGI_2. For example, it has been reported that calcium channel–blocking agents lead to a stimulation of PGI_2 production [44]. Alternatively, the adverse effects of various nonsteroidal anti-inflammatory drugs may, in part, be related to an inhibition of PGI_2 formation and absence of its cytoprotective functions.

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References

1. Mundy AR, Bewick M, Moncada S, Vane JR: Experimental assessment of prostacyclin in the harvesting of kidneys for transplantation. *Transplantation* 30:251–255, 1980
2. Haisch CE, Deepe RM, Hall WR, Park HK, Larkin EW, Thomas FT: Protective effects of prostacyclin in acute renal ischemia. *Surg Forum* 34:13–14, 1983
3. Isenberg GA, Hasday K, Oh J, Racelis D, Shapira Z, Schanzer H: Prevention of ischemic acute tubular necrosis with tris buffer or prostacyclin infusion. *Mt Sinai J Med* 48:142–145, 1981
4. Isenberg GA, Racelis D, Oh J, Schanzer H: Prevention of ischemic renal damage with prostacyclin. *Mt Sinai J Med* 49:415–417, 1982
5. Lifschitz MD, Barnes JL: Prostaglandin I_2 attenuates ischemic acute renal failure in the rat. *Am J Physiol* 247 (Renal Fluid Electro Physiol 16):F714–F717, 1984
6. Lelcuk S, Alexander F, Kobzik L, Valeri CR, Shepro D,Hechtman HB: Prostacyclin and thromboxane A_2 moderate postischemic renal failure. *Surgery* 98:207–212, 1985
7. Gerber JG, Branch RA, Nies AS, Gerkens JF, Shand DG, Hollifield J, Oates JA: Prostaglandins and renin release: II. Assessment of renin secretion following infusion of PGI_2, E_2 and D_2 into the renal artery of anesthetized dogs. *Prostaglandins* 15:81–88, 1978
8. Bolger PM, Einser Gm, Ramwell PW, Slotkoff LM, Corey EJ: Renal action of prostacyclin. *Nature* 271:467–469, 1978
9. Hashimoto, T: Effects of prostaglandin E_2, I_2 and $F_2\alpha$ on systemic and renal hemodynamics, renal function and renal secretion in anesthetized dogs. *Jap J Pharmacol* 30:173–186, 1980.
10. Baer PG, McGiff JC: Comparison of effects of prostaglandin E_2 and I_2 on rat renal vascular resistance. *Eur J Pharmacol* 54:359–363, 1979
11. Gerber JG, Nies AS: The hemodynamic effects of prostaglandins in the rat: Evidence for important species variation in renovascular responses. *Circ Res* 44:406–410, 1979
12. Baer PG, Kauker ML, McGiff JC: Prostacyclin effects on renal hemodynamic and excretory functions in the rat. *J Pharmacol Exp Ther* 208:294–297, 1979
13. Jackson EK, Heidemann HT, Branch RA, Gerkens JF: Low dose intrarenal infusion of PGE_2, PGI_2 and 6-Keto-PGE_1 vasodilate the in vivo rat kidney. *Circ Res* 51:67–72, 1982
14. Jackson EK, Heizer WA, Zimmerman JB, Branch RA, Oates JA, Gerkens JF: 6-Keto-prostaglandin E_1 is more potent that prostaglandin I_2 as a renal vasodilator and renin secretagogue. *J Pharmacol Exp Ther* 216:24–27, 1981
15. Yoshioka T, Yared A, Miyazawa H, Ichikawa I: In vivo influence of prostaglandin I_2 on systemic and renal circulation in the rat. *Hypertension* 7:867–872, 1985
16. Schor N, Brenner BM: Possible mechanism of prostaglandin–induced renal vasoconstriction in the rat. *Hypertension* 3(suppl II):II-81–II:85, 1981
17. Sullivan BA, Hak LJ, Grossman SH, Finn WF: Comparative effects of prostacyclin infusion and aortic constriction on renal vascular resistance in the rat. (abstract) *Fed Proc* 44:1103, 1985
18. Arendshorst WJ, Finn WF, Gottschalk CW: Autoregulation of blood flow in the rat kidney. *Am J Physiol* 228:127–133, 1975
19. Chasson AL, Grady HJ, Stanley MA: Determination of creatinine by means of automatic chemical analysis. *Am J Clin Pathol* 35:83–88, 1961
20. Zager RA, Timmerman TP, Merola AJ: Effects of immediate blood flow enhancement on the postischemic kidney: Functional, morphologic, and biochemical assessments. *J Lab Clin Med* 106: 360–368, 1985
21. Beierwaltes WH, Schryver S, Sanders E, Strand J, Romero JC: Renin release selectively stimulated by prostaglandin I_2 in isolated rat glomeruli. *Am J Physiol* 243:F276–F283, 1982
22. Mason J, Torhorst J, Welsch J: Role of the medullary perfusion defect in the pathogenesis of ischemic renal failure. *Kidney Int* 26:283–293, 1984
23. Mason J, Welsch J, Torhorst J: Protection from ischemic, acute renal failure —The contribution of vascular decongestion. (abstract) *Kidney Int* 27:234, 1985
24. Lefer Am, Ogletree ML, Smith JB, Silver MJ, Nicolaou KC, Barnette WE, Gasic GP: Prostacyclin: A potentially valuable agent for preserving myocardial tissue in acute myocardial ischemia. *Science* 200:52–54, 1978
25. Robert A: Cytoprotection by prostaglandins. *Gastroenterology* 77:761–767, 1979
26. Miller TA, Jacobson ED: Gastrointestinal cytoprotection by prostaglandins. *Gut* 20:75–87, 1979
27. Blackwell GJ, Radomski M, Vargas JR, Moncada S: Prostacyclin prolongs viability of washed human platelets. *Biochem Biophys Acta* 718:60–65, 1982
28. Vargas JR, Radomski M, Moncada S: The use of prostacyclin in the separation from plasma and washing of human platelets. *Prostaglandins* 23:929–945, 1982
29. Sikujara O, Monden M, Toyoshima K, Okamura J, Kosaki G: Cytoprotective effect of prostaglandin I_2 on ischemia–induced hepatic cell injury. *Transplantation* 36:238–243, 1983
30. Divald A, Ujhelyi E, Jeney A, Lapis K, Instititoris L: Hepatoprotective effects of prostacyclins on CCl_4-induced liver injury in rats. *Exp Mol Pathol* 42:163–166, 1985
31. Gabryelewicz A, Dlugosz J, Brzozowski J, Musiatowicz B, Sidun–Kurylowicz Z, Wereszczynska U, Triebling A: Prostacyclin: Effect on pancreatic lysosomes in acute experimental pancreatitis in dogs. *Mt Sinai J Med* 50:218–224, 1983
32. Mozsik G, Moron F, Fiegler M, Javor T, Nagy L, Patty I, Tarnok F: Interrelationships between the membrane–bound ATP-dependent energy systems, gastric mucosal damage produced by NaOH, hypertonic NaCl, HCl and alcohol, and prostacyclin–induced gastric cytoprotection in rats. *Prostaglandins Leukotrienes Med* 12:423–436, 1983
33. Zsoldos T, Czegledi B, Tigyi A, Javor T, Mozsik GY: Interrelationships between the development of the gastric cytoprotective effects of prostacyclin, atropine, cimetidine and the gastric

mucosal superoxide dismutase activity in rats. *Acta Physiol Hung* 64:325–330, 1984

34. SMITH EF III, GALLENKAMPER W, BECKMAN R, THOMSEN T, MANNESMANN G, SCHROR K: Early and late administration of a PGI_2-analogue, ZK 36 374 (iloprost): Efects of myocardial preservation, collateral blood flow and infarct size. *Cardiovas Res* 18: 163–173, 1984
35. THIEMERMANN C, STEINHAGEN–THIESSEN E, SCHROR K: Inhibition of oxygen–centered free–radical formation by the stable prostacyclin–mimetic iloprost (ZK 36 374) in acute myocardial ischemia. *J Cardovasc Pharmacol* 6:365–366, 1984
36. ROMSON JL, HOOK BG, KUNKEL SL, ABRAMS GD, SCHORK MA, LUCCHESI BR: Reduction of the extent of ischemic myocardial injury by neutrophil depletion in the dog. *Circulation* 67:1016–1023, 1983
37. FANTONE JC, KINNES DA: Prostaglandin E_1 and prostaglandin I_2 modulation of superoxide production by neutrophils. *Biochem Biophy Res Comm* 113:506–512, 1983
38. NAYLER WG, PURCHASE M, DUSTING GJ: Effect of prostacyclin infusion during low–flow ischaemia in the isolated perfused rat heart. *Basic Res Cardiol* 79:125–134, 1984
39. PALLER MS, HOIDAL JR, FERRIS TF: Oxygen free radicals in ischemic acute renal failure in the rat. *J Clin Invest* 74:1156–1164, 1984
40. BURKE TJ, ARNOLD PE, GORDON JA, BULGER RE, DOBYAN DC, SCHRIER RW: Protective effect of intrarenal calcium membrane blockers before or after renal ischemia. Functional morphological and mitochondrial studies. *J Clin Invest* 74:1830–1841, 1984
41. SIEGEL NJ, GLAZIER WB, CHAUDRY IH, GAUDIO KN, LYTTON B, BAUE AE, KASHGARIAN MK:Enhanced recovery from acute renal failure by the postischemic infusion of adenine nucleotides and magnesium chloride in rats. *Kidney Int* 17:338–348, 1980
42. BREZIS M, ROSEN S, SILVA P, EPSTEIN FH: Selective vulnerability of the medullary thick ascending limb to anoxia in the isolated perfused rat kidney. *J Clin Invest* 73:182–190, 1984
43. BREZIS M, SHANLEY P, SILVA P, SPOKES K, LEAR S, EPSTEIN FH, ROSEN S: Disparate mechanisms of hypoxic cell injury in different nephron segments: Studies in the isolated perfused rat kidney. *J Clin Invest* 76:1796–1806, 1985
44. FORSTER W: Significance of prostaglandins and thromboxane A2 for the mode of action of cardiovascular drugs, in *Advances of Prostaglandin and Thromboxane Research* (vol. 7) edited by SAMUELSSON B, RAMWELL PW, PAOLETTI R, New York, Raven Press, 1980, pp 609–618