Atrial Natriuretic Peptide Protects Against Cold Ischemic Injury in the Isolated and In Situ Rat Kidney

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Acute renal failure after transplantation remains a major cause of morbidity. Moreover, initial graft dysfunction may have adverse effects on ultimate graft function, especially when cyclosporine is used in the immunosuppression regimen (1,2). We have observed, as have others, that atrial natriuretic peptide (ANP) may ameliorate warm ischemic injury to the kidney (3,4). There is, however, little information concerning the protective effect of ANP against cold ischemic injury. To assess the potential usefulness of ANP as an additive to organ preservation solutions, the studies presented here were undertaken.

METHODS

Male Sprague-Dawley rats (300 to 400 g) fed ad libitum on standard rat chow (Rodent Blox; Ralston Purina, St. Louis, MO) and water were used in all studies. For isolated kidney experiments, rats were anesthetized with pentobarbital (50 mg/kg i.p.) and the right kidney was isolated, perfused with either 30 mL of control storage solution (Collins' solution) containing no additives or ANP (1 μg/30 mL) [AP-24; Merck Sharp & Dohme, Rahway, NJ] at 4°C at 100 mm Hg perfusion pressure, and stored at 4°C for 4 h. At this time, the left kidney was removed and weighed to normalize the physiologic data. After storage, kidneys were reperfused on an isolated perfused kidney (IPK) circuit for 1 h at 37°C with Krebs-Henseleit saline supplemented with 6.7% albumin, 5 mM glucose, 20 amino acids and [14C]inulin at 100 mm Hg perfusion pressure (4–6). In some experiments, ANP (1 μg/100 mL) was added to the perfusion media. Measurements of [14C]inulin and sodium concentrations in perfusate and urine, as well as calculations of renal perfusate flow (RPF), urine flow (V), inulin clearance (Cin), and net tubular sodium reabsorption (TNa+), were made as previously described (4–6). The physiologic functions of the following groups were compared during reperfusion: Group I (N = 6) kidneys, which were flushed with control flush and reperfused with control perfusate; Group II (N = 6) kidneys, which were flushed with ANP and reperfused with control perfusate; Group III (N = 6) kidneys, which were flushed with ANP and reperfused with ANP; and Group IV (N = 6) kidneys, which were flushed with control flush and reperfused with ANP.

In vivo experiments (N = 6) were also performed. In anesthetized rats, the abdomen was opened with a midline incision and both right and left kidneys were exposed. Carotid arterial and jugular venous catheters were placed as were right and left ureteral catheters (PE-50 tubing). Smaller catheters (PE-10 tubing) were inserted into the right and left femoral arteries and threaded into the ipsilateral renal artery. Both kidneys were flushed with 5% dextrose at 4°C and clamped for 1 h by using vascular clamps applied across each renal artery. One microgram of ANP was added to the flush for the right, but not for the left, kidney. The kidneys were packed in ice during the hour of ischemia. An inulin clearance determination (4) was performed for right and left kidneys at 2 h of reflow. After this study, each kidney was removed and weighed. All experiments were in accord with NIH guidelines for animal experimentation.

Statistical analysis included one-way analysis of variance and unpaired Student's t test for the IPK data, employing Scheffe's correction for multiple comparisons. For in vivo data, the Student's t test for paired data was employed. Statistical significance is reported at P < 0.05 and P < 0.01 levels.

RESULTS

The IPK data, which were averaged over 60 min of reperfusion, are summarized in Table 1. As is clear...
from these data, addition of ANP to the flush afforded marked amelioration of functional injury after cold ischemia. Compared with the function of the normal isolated rat kidney, addition of ANP to the flush restored Cin to approximately 75% of uninjured values (6). However, addition of ANP to the reperfusion media alone did not improve functional recovery. When administered to kidneys flushed with ANP, the addition of ANP to the reperfusion media did not afford any additional protection.

In the in vivo experiments, V (6.5 ± 1.2 versus 1.7 ± 0.5 μL/min/g; P < 0.01), Cin (99.6 ± 26.1 versus 22.0 ± 11.1 μL/min/g; P < 0.05), and TNa* (13.1 ± 3.9 versus 3.3 ± 1.6 μmol/min/g) were all significantly higher in the right (ANP-treated) kidney than in the left (control flush) kidney.

**DISCUSSION**

In the study presented here, ANP added to the flushing solution improved functional recovery of the kidney in both in vitro and in vivo models of cold ischemia. Thus, ANP affords a protective effect against both warm (3,4) and cold ischemia, circumstances routinely present during clinical kidney transplantation. With the cold ischemia, the protective effect is manifested primarily when the ANP is added to the storage solution and addition of ANP during the reperfusion period was not efficacious. In the study reported here, the dose of ANP which afforded protection against cold ischemia (3.3 μg/100 mL or a total dose of 1 μg to the kidney) was much lower than the ANP dose necessary to attenuate warm ischemia (100 μg/100 mL) (4). These results also demonstrate that a dose of ANP which does not affect systemic hemodynamics (3,4), given before the insult, can markedly attenuate cold ischemic injury. If these results are confirmed clinically, addition of this agent to the flushing solution administered during kidney harvesting could ameliorate initial graft dysfunction after transplantation and potentially improve long-term graft survival.

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**REFERENCES**