Effects of renal autograft ischemic storage and reperfusion on intraoperative hemodynamic patterns and plasma renin concentrations in clinically normal cats undergoing renal autotransplantation and contralateral nephrectomy

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Objective—To evaluate the effect of the duration of cold ischemia on the renin-angiotensin system during renal transplantation in cats and to define the potential influence of vasoactive factors in renal tissue following cold ischemic storage versus warm ischemic storage.

Animals—10 purpose-bred 6-month-old sexually intact female cats.

Procedures—10 cats underwent renal autotransplantation after 30 minutes (n = 5) or 3 hours (5) of simple, ex vivo cold storage of renal autographs. Following autograft reperfusion, direct hemodynamic variables were measured with a telemetric implant and samples were collected for plasma renin concentration. Activation of vascular-related genes (renin, endothelin, and angiotensin converting enzyme) relative to 2-hour simple cold or warm ischemia was also evaluated.

Results—No significant difference between groups was detected in any of the hemodynamic variables or postreperfusion plasma renin concentrations measured in this study relative to the duration of cold ischemic storage. There was also no difference between warm- and cold-stored kidneys in the expression of vascular-related genes.

Conclusions and Clinical Relevance—Prolonged renal ischemia for clinically relevant durations does not appear to predispose clinically normal cats to altered hemodynamics or high plasma renin concentrations following graft reperfusion. Activation of vasoactive genes does not appear to be influenced by type of ischemia over 2 hours. (*Am J Vet Res* 2010;71:1220–1227)

Perioperative hypertension has been reported for people[1] and cats[2,3] during and immediately after renal transplantation. In people, increased postperfusion systolic blood pressure is associated with an increased risk of acute graft rejection.[1] Other studies[4] confirm a relationship between postoperative hypertension and early acute graft rejection, especially in people that were normotensive prior to renal transplantation. Knowledge of the pathogenesis of posttransplantation hypertension may identify therapeutic targets which, in turn, may offer opportunities to minimize acute graft damage in human and veterinary patients.

One potential mechanism to explain intraoperative hypertension following renal allograft reperfusion is systemic supraphysiologic renin exposure following graft reperfusion. Renin may accumulate in the juxtaglomerular cells as a result of the lack of blood pressure during autograph storage. Upon release from the juxtaglomerular cells, renin converts angiotensinogen to angiotensin I, which is then acted on by ACE to form angiotensin II. Acutely, angiotensin II increases blood pressure by direct vasoconstriction. Chronically, angio-

Abbreviation

ACE Angiotensin converting enzyme

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tensin II promotes renal retention of sodium and water through a direct action on the kidney and stimulation of the adrenal glands to produce aldosterone that, in turn, increases distal renal tubule reabsorption of sodium and water.

In a previous report[2] of 8 cats with chronic renal failure receiving cold-stored allografts, severe hypertension immediately following graft reperfusion was identified in 3 cats. These 3 cats had an approximate maximum Doppler blood pressure measurement between 190 and 250 mm Hg within 5 to 25 minutes after release of vascular clamps and graft reperfusion.[2] This temporal relationship supports the described hypothetical mechanism. Further, supraphysiologic renin exposure has been suggested as a cause of hypertension following renal transplantation in rats.[5] Although definitive evidence of this mechanism has not been established, a 74% increase in kidney tissue renin concentration has been documented for rats following reperfusion after 1 hour of renal in vivo warm ischemia, compared with that of sham-operated control rats.[6] In the same study,[6] urine concentrations of angiotensin I and tissue concentrations of angiotensin II were also found to increase following renal ischemia. The consequences of renal ischemia on the renin-angiotensin system in cats are unknown.

In the clinical setting, renal harvest and transplantation in cats are performed both with and without the use of cold ischemic treatment of the donor kidney, depending on surgeon preference.[2,7] Introduction of a cold ischemic period allows for sequential harvest and transplantation surgeries by storing the kidney during the surgical closure of the donor and anesthetic induction and vessel preparation of the recipient. The effect of the duration of cold ischemia on the production of and systemic exposure to renin and on hemodynamics after reperfusion in cats is unknown.

The purposes of the study reported here were to evaluate the effect of the duration of cold ischemia on the renin-angiotensin system during renal transplantation in cats and to define the potential influence of vasoactive factors in renal tissue following cold ischemic storage versus warm ischemic storage. Our hypothesis was that a longer duration of cold ischemia will result in increased intraoperative blood pressure following autograft reperfusion, compared with immediate transplantation, and that changes in blood pressure would be associated with an increase in plasma renin concentrations. Also, we hypothesized that cold storage of renal autographs would result in less activation of vascular-related genes for renin, endothelin, and ACE, compared with that of renal autographs stored in warm ischemic conditions.

Materials and Methods

Animals—All procedures were approved by the Institutional Animal Care and Use Committee at the University of Georgia. Ten purpose-bred 6-month-old sexually intact female cats were used in the study. The general health of the cats was confirmed on the basis of findings on physical examination, CBC, serum biochemical analysis, and urinalysis prior to the study.

Anesthesia—Cats were premedicated with acepromazine (0.001 mg/kg, IM), buprenorphine (0.03 mg/kg, IM), and ketamine (7 mg/kg, IM) 30 minutes prior to induction of anesthesia. General anesthesia was induced with isoflurane in 100% oxygen delivered by face mask. When an appropriate depth of anesthesia was attained, cats were endotracheally intubated and maintained on isoflurane. Cats received additional doses of buprenorphine (0.03 mg/kg, IV) every 6 hours. All cats received crystalloid fluids IV at a rate of 10 mL/kg/h for the first hour, followed by 5 mL/kg/h thereafter. Hetastarch was administered (10 mL/kg) to all cats during the course of the anesthetic period. Body temperature was supported with a hot-water blanket and a hot-air patient warming system.

Depth of anesthesia was monitored by evaluation of eye position, jaw tone, heart rate, respiratory rate, and blood pressure. Anesthesia and fluid rate were adjusted to maintain a mean blood pressure > 60 mm Hg. Cats were kept at a surgical plane of anesthesia (stage III, medium plane)[8] except during periods of cold storage of renal autographs when cats were kept at a lighter plane of anesthesia (stage III, light plane).

Telemetry catheter system—Approximately 1 week prior to renal transplantation, radiotelemetry catheters[a] were implanted in the right carotid artery.[9] Cats were anesthetized with the regimen already described. The transmitter portion of the catheter was sutured to the musculature of the ventral portion of the neck. Postoperatively, a transdermal fentanyl patch (25 µg/h) was applied to each cat. Cats were monitored twice daily for signs of discomfort and to assess appetite, thirst, urination, and defecation. Analgesia was augmented with hydromorphone (0.1 mg/kg, SC) as needed on the basis of a discomfort score (**Appendix**). Cats were housed individually, fed standard diets, and given water ad libitum.

Each cage was equipped with 3 radiotelemetry receivers.[b] Animal data, along with ambient barometric pressure data,[c] were routed through two 20-channel matrices[d] to a dedicated computer. Pulse rate, pulse pressure, and systolic, mean, and diastolic blood pressure data were acquired and stored every 5 minutes with a software program.[e] These data were used to establish a preoperative baseline.

Renal autotransplantation—All cats underwent a standard heterotopic renal autotransplantation and contralateral nephrectomy. The left kidney was autotransplanted preferentially; however, if a double renal artery was encountered on the left side, a right kidney was transplanted instead. A standard nephrectomy was performed on the kidney that was not transplanted. After removal, the autograft was flushed with a cold (approx 4°C) sucrose phosphate solution[2] until the renal parenchyma was uniformly blanched and the renal vein effluent was clear. The autograft was then placed in cold (approx 4°C) sucrose phosphate solution in a sterile bowl surrounded by a frozen saline (0.9% NaCl) solution slush and transplanted after a 30-minute simple cold ischemic period (30-minute ischemia group; n = 5) or transplanted after approximately 3 hours of simple cold storage of the renal autograph (3-hour isch-

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emia group; 5). Assignment to groups was randomized. The vascular anastomoses were performed with an end-to-side technique. The caudal vena cava was partially occluded, and the renal vein was sutured with 10-0 polyester in 2 simple continuous suture lines. Next, the aorta was clamped, an aortotomy was performed, and the renal artery was sutured with 9-0 nylon in 2 simple continuous suture lines. If needed on the basis of subjective evaluation of the appearance of the autograft and the renal artery, acepromazine (0.025 to 0.05 mg) was used topically to control arterial vasospasm. The ureteral papillae were implanted as described previously.[10]

Intraoperative hemodynamic monitoring—From the time of anesthetic induction to endotracheal extubation, systolic, diastolic, and mean blood pressures; pulse pressure; and pulse rate were measured and recorded every 5 minutes by use of the telemetric catheter system. Intraoperative hypertension was defined as systolic blood pressure that was > 180 mm Hg from the period of allograft reperfusion to endotracheal extubation; each measurement was taken for > 60 seconds. Cats were given hydralazine SC to effect (1 mg, then 1.5 mg, then 2.5 mg) if systolic blood pressure was ≥ 180 mm Hg. Hydralazine was given to prevent hypertensive complications as part of a longer-term study.[9] Mean hemodynamic variables from the beginning of surgery to the start of anastomosis were calculated as baseline 1. Mean variables during the time of anastomosis were calculated as baseline 2. Cats in the 3-hour ischemia group remained anesthetized and were anephric during cold storage of the renal autograph. Variables following reperfusion were compared as 15-minute mean values between groups as raw data and percentage change from baselines 1 and 2. Preoperative baselines were also compared between groups.

Real-time quantitative PCR assays—After the nontransplanted kidney was removed, it was flushed as described for the autograft. The kidney was then divided transversely. Samples of the renal cortex were obtained at this time for baseline measurements. Half of the kidney was placed in an approximately 4°C sucrose phosphate solution (cold storage), and the other half was stored in the abdomen between the liver and diaphragm (warm storage). After 2 hours of storage, a second sample of renal cortex was obtained from each kidney half and placed immediately in a tissue storage reagent[f] to prevent degradation of RNA. Per the manufacturer's instructions, samples were refrigerated (2.5° to 5.5°C) overnight and then stored at –80°C until evaluation.

Tissue samples were thawed at room temperature (approx 20° to 22°C), minced with a scalpel blade, and processed with a homogenizer. Tissue was then subjected to 1 freeze-thaw cycle and multiple passages through a 22-gauge needle. The RNA from the tissue was isolated by use of an RNA assay kit[g] according to the manufacturer's protocol and treated by incubation with DNase I at 24°C for 30 minutes. Only samples having a 260:280 nm absorbance ratio between 2.0 and 2.2, as measured with a spectrophotometer, were processed for cDNA synthesis by use of a high capacity cDNA kit[h] with 100 ng of RNA as the template. Real-time quantitative PCR assays, with SYBR green as a detector, were performed with 18S rRNA assays[i] serving as endogenous control samples. Conditions for amplification were 2 minutes at 50°C; 10 minutes at 95°C, followed by 40 cycles of 15 seconds at 95°C; and 60 seconds at 60°C. This was followed by a dissociation stage for 15 seconds at 95°C to ensure the presence of a single amplicon. Five genes of interest were evaluated in the samples (ie, renin, ACE 1 and 2, and endothelin 1 and 2). Oligonucleotide primers used for the detection of cDNA specific for these feline proteins were derived from gene sequences in the GenBank database[j] and designed by use of a software program[k] (**Table 1**). Dissociation curve analysis and agarose gel electrophoresis revealed single products for all primers; the PCR amplification efficiency was approximately 100%, and amplification efficiencies of the target and reference (18S rRNA) were equivalent. The PCR assays contained 300nM of each primer, master mix, and 2 µL of the diluted cDNA sample in a final volume of 10 µL. A final amount of 3 ng of cDNA template was used for each assay. Intra-assay variation was evaluated by use of a pooled equine cDNA (ie, control cDNA) sample prepared from RNA of cells stimulated with *Escherichia coli* O111:B4 lipopolysaccharide (100 pg/mL). Changes in mRNA expression were calculated by relative quantification against 18S rRNA of the gene of interest with the $\Delta\Delta C_T$ method by use of the following equation:

$$\Delta\Delta C_T = ([\text{gene } C_T - 18S \text{ rRNA } C_T]_{\text{warm or cold}} - [\text{gene } C_T - 18S \text{ rRNA } C_T]_{\text{baseline}})$$

where C_T is defined as the amplification cycle at which the gene reached a threshold level of template concentration. Fold changes in gene expression were calculated as $2^{-\Delta\Delta CT}$. Results were expressed as the mean fold change in gene expression at each time point. All reactions were performed in triplicate wells.

Fluorometric plasma renin concentration assay—Immediately after induction of anesthesia, a single lumen catheter was inserted into the left jugular vein.

Table 1—Primer sequences designed for use in this study for the detection of cDNA of specific feline proteins.

Gene	Forward primer	Reverse primer
Renin	5′-ACC CCT TGG GAG AGG ATG T G-3′	5′-TTC ATG CTG GCC AAG TTT GA-3′
ACE I	5′-ATC TGG AAC ACC TCT ACC ATC AAG T-3′	5′-TCC CCT GAG GTT GAT GTA TCT GT-3′
ACE II	5′-GAA GGT CCC CTG CAC AAA TG-3′	5′-GCT AAG GTC CAG GGC TTT GA-3′
Endothelin I	5′-AAC ACT CCC GAG CAC ATC GT-3′	5′-GGC TGG CAC ACT GAC ATC TG-3′
Endothelin II	5′-CAG CCA AGA GCC ACT TTG C-3′	5′-GCT CCT TCC CAG CAT TCC TT-3′

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Two milliliters of blood was collected via this catheter immediately after placement. This sample was labeled as baseline. Following reperfusion of the autograft, 2 mL of blood was obtained at 5, 15, 30, 60, and 120 minutes. Blood was collected into tubes containing EDTA and stored on ice. Plasma was obtained from the blood sample within 30 minutes of collection and stored at –80°C until analysis.[9]

A 5-carboxyfluorescein–based intramolecularly quenched fluorescence resonance energy transfer peptide that is cleaved by renin[l] was used to evaluate plasma renin concentration. This renin substrate releases a green fluorescent product upon cleavage by renin. The 5-carboxyfluorescein is monitored for excitation and emission at 490 ± 20 nm and 520 ± 20 nm, respectively. All samples were evaluated in triplicate on a microplate fluorometer coupled with a dedicated computer and software system. This fluorescence resonance energy transfer peptide is used to measure in vitro renin inhibition and was modified and optimized to measure plasma renin concentration in cats.[11]

A 5-carboxyfluorescein standard[m] was used to confirm fluorometer function and sufficient signal. By use of 10 µL of a 1:50 dilution of the substrate in buffer and 90 µL of fetal bovine serum,[n] a standard curve of renin concentration was plotted on the basis of known serial dilutions (20, 10, 5, 2.5, 1.25, 0.625, and 0 ng/mL) of recombinant human renin.[o] A standard curve was calculated for each vial of substrate. A regression line was fitted to the curve, and the slope and R^2 value were calculated. In 96-well, clear, flat-bottom microplates, 90 µL of plasma was combined with 10 µL of renin substrate. Plates were centrifuged at 3,000 × *g* for 2 minutes and incubated at 37°C for 4 hours. Known renin concentrations and a negative control were evaluated on each fluorometry plate. Based on the regression equation from the control samples, the relative fluorometric units were converted into ng/mL.

Statistical analysis—All data were analyzed with a software package.[p] Data were analyzed for normality by use of the Kolmogorov-Smirnov test. Mean baseline raw hemodynamic data (pulse rate; mean, systolic, and diastolic blood pressures; and pulse pressure) and intraoperative, 15-minute means of raw hemodynamic data (pulse rate; mean, systolic, and diastolic blood pressures; and pulse pressure) at 15, 30, 45, 60, 75, and 90 minutes after autograft reperfusion as well as percentage change in intraoperative values from baseline 1 and baseline 2 were compared between the 30-minute and 3-hour ischemia groups with a Wilcoxon matched-pairs rank test. Approximate plasma renin concentration, anastomosis time, cat body weight, and values for vascular-related genes expression were compared with a Student paired *t* test. A repeated-measures ANOVA was used to compare approximate plasma renin concentrations following graft reperfusion with plasma renin concentrations at baseline. Significance for tests was set at a value of $P < 0.05$ (2 tailed). Data are reported as mean ± SEM.

Results

Mean body weight of the cats was 2.53 ± 0.07 kg. There was no significant ($P = 0.27$) difference in body weight between groups.

Perioperative blood pressure—All cats survived the telemetric catheter placement, and there were no complications with the catheters for the duration of the study. There was no significant difference in preoperative baseline variables. The right kidney was transplanted in 3 cats (2 cats in the 30-minute ischemia group and 1 cat in the 3-hour ischemia group) because of double left renal arteries. All cats survived the transplantation surgery, and there were no clinically important perioperative complications.

The mean anastomosis time was 84.0 ± 5.9 minutes in the 30-minute ischemia group and 76.6 ± 5.5 minutes in the 3-hour ischemia group; these values were not significantly ($P = 0.3$) different. The mean duration of cold storage of renal autographs was 27.2 ± 1.9 minutes in the 30-minute ischemia group and 188.6 ± 5.2 minutes in the 3-hour ischemia group. No significant differences were detected when raw hemodynamic data or data expressed as a percentage change from baseline 1 or baseline 2 were compared between groups (**Table 2**). Two cats, 1 in each group, were given hydralazine SC at approximately 80 and 95 minutes, respectively, after autograft reperfusion. One of these 2 cats had a right kidney transplanted; the other had a left kidney transplanted. Each in-

Table 2—Mean ± SEM hemodynamic values following 30-minute or 3-hour cold storage of renal autographs and renal autotransplantation 10 in clinically normal cats.

	30-minute ischemic group (n = 5)					3-hour ischemic group (n = 5)				
Time points	**PR (beats/min)**	**dBP (mm Hg)**	**mBP (mm Hg)**	**sBP (mm Hg)**	**PP (mm Hg)**	**PR (beats/min)**	**dBP (mm Hg)**	**mBP (mm Hg)**	**sBP (mm Hg)**	**PP (mm Hg)**
Before surgery	195.1 ± 10.3	82.3 ± 2.1	97.9 ± 2.2	109.7 ± 2.4	27.4 ± 1.0	180.8 ± 11.9	79.4 ± 0.9	96.3 ± 1.3	110.0 ± 2.0	30.6 ± 1.1
Baseline 1	174.5 ± 10.3	65.2 ± 5.2	81.6 ± 7.3	97.4 ± 8.7	32.2 ± 4.2	182.0 ± 6.9	58.8 ± 4.0	74.0 ± 3.5	92.2 ± 5.6	33.3 ± 2.8
Baseline 2	193.0 ± 12.5	61.4 ± 3.3	76.3 ± 4.6	90.3 ± 5.7	28.9 ± 3.3	198.9 ± 9.6	58.9 ± 3.9	79.0 ± 4.4	97.6 ± 5.1	38.7 ± 3.4
After perfusion										
15 min	194.9 ± 14.0	53.0 ± 6.3	67.2 ± 7.15	82.7 ± 8.7	29.6 ± 2.9	183.2 ± 12.1	46.7 ± 5.8	64.6 ± 6.9	82.5 ± 7.4	35.9 ± 2.9
30 min	190.6 ± 17.6	51.9 ± 3.1	67.2 ± 2.5	84.3 ± 2.6	38.6 ± 6.4	184.2 ± 13.6	51.0 ± 4.0	71.2 ± 5.0	93.1 ± 5.8	41.2 ± 2.9
45 min	193.4 ± 18.3	55.6 ± 4.0	71.8 ± 5.0	89.0 ± 5.7	33.3 ± 4.2	183.4 ± 12.7	50.1 ± 3.7	70.5 ± 4.7	93.1 ± 5.7	43.0 ± 3.6
60 min	193.8 ± 20.0	50.2 ± 2.8	65.7 ± 2.2	82.9 ± 1.5	32.7 ± 3.4	187.7 ± 13.1	53.2 ± 4.9	74.0 ± 5.5	97.6 ± 5.6	44.4 ± 3.8
75 min	188.1 ± 22.9	51.1 ± 3.0	67.3 ± 2.7	84.8 ± 2.5	33.8 ± 4.4	182.3 ± 16.2	46.1 ± 3.7	65.5 ± 3.1	91.9 ± 3.0	45.8 ± 4.0
90 min	190.9 ± 23.3	54.7 ± 1.1	71.6 ± 1.5	89.4 ± 3.7	34.7 ± 4.6	174.6 ± 11.7	45.1 ± 3.9	66.1 ± 2.9	94.9 ± 3.4	49.7 ± 5.8

Baseline 1 = Data obtained from the time of induction of anesthesia to the beginning of vascular anastomosis. Baseline 2 = Data obtained during vascular anastomosis. dBP = Diastolic blood pressure. mBP = Mean blood pressure. PP = Pulse pressure. PR = Pulse rate. sBP = Systolic blood pressure.

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Table 3—Real-time quantitative PCR assay results in renal tissue following 2 hours of warm or cold ischemia.

Gene	Storage condition	$\Delta\Delta C_T$	Fold change ($2^{-\Delta\Delta C_T}$)	*P* value
Renin	Warm	–0.022	1.02	0.43
	Cold	0.103	0.93	
ACE I	Warm	–0.599	1.51	0.81
	Cold	–0.482	1.40	
ACE II	Warm	–0.273	1.21	0.65
	Cold	0.074	0.95	
Endothelin I	Warm	–0.259	1.20	0.85
	Cold	–0.183	1.14	
Endothelin II	Warm	–0.342	1.2	0.25
	Cold	0.096	0.94	

C_T = Amplification cycle at which the gene reached a threshold level of template concentration.
$\Delta\Delta C_T = ([\text{gene } C_T - \text{18S rRNA } C_T]_{\text{warm or cold}} - [\text{gene } C_T - \text{18S rRNA } C_T]_{\text{baseline}})$.

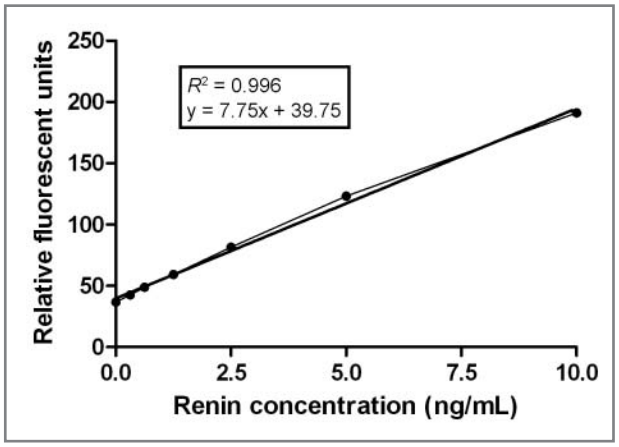

Figure 1—Standard curve for semiquantitative assessment of feline plasma renin concentration. Known concentrations of recombinant human renin were incubated in 90 µL of fetal bovine serum with 10 µL of a 1:50 dilution of fluorometric substrate for 4 hours at 37°C. The slope of the regression line was used to calculate expected renin concentration on the basis of relative fluorescent units obtained from biological samples.

stance of hydralazine administration was after completion of surgery and approximately 5 to 10 minutes prior to extubation.

Real-time quantitative reverse transcription PCR assay—All primers were successfully designed and validated for use in a SYBR green assay. No significant differences were observed for any gene in warm- or cold-stored renal cortex, compared with control values (**Table 3**).

Fluorometric plasma renin concentration assay—A standard curve was constructed, revealing a linear relationship between known renin concentration and relative fluorescence units (**Figure 1**). The baseline renin sample was taken after induction of anesthesia and placement of a catheter in the jugular vein. The mean time from the baseline renin measurement to the first renin measurement following reperfusion (5 minutes after graft reperfusion) was 6.3 ± 0.1 hours in the 3-hour ischemia group and 4.4 ± 0.5 hours in the 30-minute ischemia group. No significant difference was observed in the plasma concentration of renin at

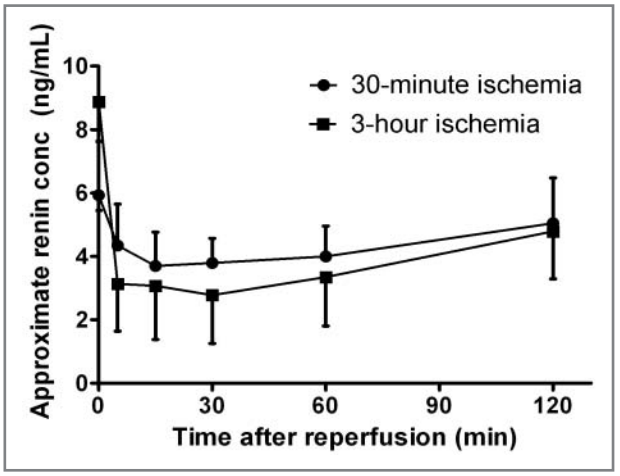

Figure 2—Mean ± SEM of plasma renin concentration (conc) following renal autograft reperfusion in cats after 30 minutes (n = 5; circles) or 3 hours (5; squares) of simple cold (approx 4°C) ischemic storage and contralateral nephrectomy. The baseline sample (time 0) was after anesthesia induction and placement of a catheter in the jugular vein. The remaining samples were taken following isograft reperfusion. The mean time from the baseline renin sample to the first renin sample (5 minutes) was 6.3 ± 0.12 hours in the 3-hour ischemia group and 4.42 ± 0.50 hours in the 30-minute ischemia group. There is no significant difference between groups regarding plasma renin concentration at any time after reperfusion. Compared with baseline, there was no significant difference in renin concentrations over time in the 30-minute ischemia group; however, in the 3-hour ischemia group, all reperfusion values were significantly ($P < 0.01$) less than baseline values.

any point measured during this study between groups (**Figure 2**). Compared with baseline, there was no significant difference in renin concentrations over time in the 30-minute ischemia group; however, in the 3-hour ischemia group, all reperfusion values were significantly ($P < 0.01$) less than baseline values.

Discussion

We used 2 strategies to characterize the influence of type and duration of ischemic storage on the renin angiotensin system in feline kidneys. The first phase of the study demonstrated no significant difference in intraoperative hemodynamic variables and plasma renin concentrations following renal autotransplantation and contralateral nephrectomy after 30 minutes or 3 hours of simple ex vivo cold storage of renal autographs. The second phase of the study confirmed a lack of quantitative change in renin, ACE, or endothelin gene expression in kidney tissue following 2 hours of warm or cold ischemic storage. Therefore, in clinically normal cats, cold ischemia of kidneys of 3 hours does not appear to alter hemodynamics after reperfusion or the renin-angiotensin system, compared with a 30-minute cold ischemic period.

Clinically, hypertension in cats after renal transplant is commonly viewed as a postoperative management problem,[3] and its occurrence during the intraoperative period was rare when hypertensive feline transplant recipients that had received preoperative antihypertensive treatment were retrospectively reviewed. Out of 30 feline transplant recipients receiving grafts with no cold ischemic period, 2 (7%) developed hypertension dur-

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ing surgery.[12] However, following variable durations of simple cold ischemic storage in sodium gluconate storage solution or phosphate-buffered sucrose solution, 3 of 8 cats became acutely hypertensive intraoperatively immediately after graft reperfusion and 2 of those cats had unfavorable outcomes.[2] There are significant differences in the protocols of these studies,[2,12] including preoperative antihypertensive treatment, different flushing solutions, different storage times, and different ischemic variables; thus, the specific reason for the apparent discrepancy is unknown.

It was our hypothesis that following a period of warm or cold ischemia renal cortical tissue would have an increased level of renin gene expression. This was based on experiments evaluating renal ischemia in rats and dogs, where renal cortical and plasma renin concentration (ng of angiotensin•I $mg^{-1}•h^{-1}$) increased after in vivo, warm ischemia and reperfusion.[6,13] Also in rats, after warm ischemia and reperfusion, ACE concentration (nmol of histidine-leucine•$mg^{-1}•min^{-1}$) decreased, compared with a sham-operated control.[6] On the basis of our data, it appears in cats there is no increase in cortical renin or ACE gene expression during periods of warm or cold ischemia. Because renin is stored in an inactive form within cytoplasmic granules of juxtaglomerular cells,[14] there may not be a substantial drive to initiate transcription in this situation and time frame. Quantitative changes in mRNA may therefore be dependent on graft reperfusion and depletion of cytoplasmic stores.

Endothelin is a powerful vasoactive peptide released from endothelial cells after injury.[15–18] Endothelin has been demonstrated to play a role in a variety of renal disease states.[17,18] Blockade of endothelin receptors has proven to have a protective effect after ischemia-reperfusion injury in experimental renal transplantation, supporting a contributing role in the pathogenesis of chronic kidney disease.[15,16] Endothelin production is regulated at the level of transcription and is stimulated by other vasoconstrictor molecules (eg, angiotensin II and prostaglandin), inflammatory cytokines (transforming growth factor-β and interleukin-1), and physical factors (mechanical strain and low shear stress).[17] Ischemia alone, either warm or cold, did not result in a significant change in endothelin mRNA in this study. Reperfusion may be necessary to sufficiently damage endothelial cells and cause a measurable effect, and measurement of vasoactive compounds, like endothelin, after a period of reperfusion should be the focus of future studies.

Hypertension in cats may not result from a local (ie, renal graft) stimulus. Although they were not studying cats with naturally occurring kidney disease, other investigators have observed either hypertension with neurologic clinical signs[19] or neurologic clinical signs after renal transplant, which presumably may have been related to hypertension,[20] in clinically normal cats. Although hypertension was not documented for all cats, each case had histologic evidence of systemic thromboembolic disease. One study[19] found multifocal microemboli throughout the lungs and brainstem in a cat that became very hypertensive (systolic blood pressure, 220 mm Hg) and had a head tilt with rotary nystagmus following renal transplantation. In the other study,[20] 2 cats developed seizures after transplantation and had evidence of thrombi in the autografts, adrenal glands, and lungs. Blood pressure around the time of the seizures was not reported in the latter study. It may be hypertensive episodes and their associated neurologic sequelae result from a centrally mediated etiology secondary to thromboembolic pathological changes. This suggests that in some instances, posttransplantation hypertension may be a centrally mediated phenomenon, and magnetic resonance imaging evaluation of the brain of severely hypertensive transplant recipients may be an interesting future diagnostic strategy.

The assay used to quantify renin concentration in this study was modified to measure feline renin in plasma.[11] The assay is designed to measure inhibition of recombinant human renin in vitro. Although the commercially available substrate is designed to be cleaved by recombinant human renin, angiotensinogen is conserved across many taxa, especially in the region adjacent to renin's cleavage point.[21] This conservation and our ability to demonstrate fluorescent signal in feline plasma suggest that feline renin is able to cleave the fluorometric substrate and provide a semiquantitative measure of plasma renin concentration. The results of the assay are qualitatively supported by the minimal changes in postreperfusion blood pressure.

Although the data generated from our study of ischemic storage support the conclusions that supraphysiologic renin release does not occur following cold renal ischemia, it should also be recognized that the experimental conditions do not reflect the clinical situation where the recipient animal has chronic renal disease. In 1 case series,[22] the mean age of feline transplant recipients was 8 years, which is significantly greater than the cats in the study reported here. Additionally, the cardiovascular environment brought about by chronic renal disease is dramatically different, compared with the healthy 6-month-old cats in the study reported here.[23] There is clear evidence that people with chronic renal disease have increased markers of systemic oxidative stress (plasma protein carbonyl group content, plasma free F2-isoprostane content, and plasma protein reduced thiol content) and acute inflammation (C-reactive protein and interleukin-6), compared with healthy people.[24] Although the effect of inflammation on the pathogenesis of chronic renal disease in cats has not been established, on the basis of studies in people, there is evidence the vasculature may be dysfunctional. It has been demonstrated in a small number of cats with chronic renal disease that the renin-angiotensin-aldosterone system is significantly upregulated.[25] This may result in a substantial amount of renin systemically available and increase the likelihood of hypertension in cats with chronic renal disease. Chronic renal disease may also result in endothelial cell damage with impaired vasodilator synthesis[26] or electrolyte abnormalities with associated vasoactive mediator alterations.[27] Both of these consequences of kidney disease may alter the vascular response to renal transplantation and potentially augment a systemic response to vasoactive compounds.

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Other potential differences existed between our study and conditions observed in with cats with naturally occurring chronic renal disease that received a renal transplantation. Mean anastomosis time in this set of experiments was longer than ideal[2,28] (80.3 minutes vs < 60 minutes). This allowed the graft to experience a prolonged period of secondary cold ischemia as the graft slowly warmed as a result of exposure to ambient conditions. Should ischemia-reperfusion injury play a critical role in intraoperative hypertension, it should have been exacerbated by this insult. Although warm ischemia has been linked to chronic hypertension in people,[29] there is no evidence that it is involved in acute hypertension.

Although there were no significant differences between the experimental groups at any time, the 3-hour ischemia group did have a significant reduction in approximate plasma renin concentration after kidney reperfusion, compared with the baseline concentration. A single baseline measurement was taken in each cat after anesthetic induction and placement of a catheter in the jugular vein. A single baseline sample was taken to minimize sampling blood loss in anticipation of the possibility of anastomotic hemorrhage and the need for multiple postreperfusion samples. The reason for the decrease in renin concentration, compared with baseline, is unknown but may relate to expansion of vascular volume as a result of IV anesthetic fluid administration. Additionally, during the cold ischemic period, the cats were anephric. Lack of renal tissue will result in reduced plasma renin concentration.[30] Therefore, cats undergoing a longer anephric period will likely have a greater reduction in plasma renin concentration.

Because of the necessity to undergo renal harvest, cold ischemia time, and transplantation, anesthesia time in these cats was quite prolonged. The renin-angiotensin-aldosterone system should remain functional under general anesthesia.[31] Every attempt was made to standardize anesthetic variables in all cats. However, given individual variability in cats and the duration of anesthesia experienced by these cats, one cannot completely standardize the entire anesthetic experience. Renin concentration under anesthesia will depend upon several factors, including extracellular fluid osmolality and sodium concentration, blood volume, catecholamine release secondary to surgical pain, and arterial blood pressure.[32] A previous study,[6] which documented increased renin concentration in rats following in vivo renal ischemia, utilized pentobarbital sodium for anesthesia. Although pentobarbital does affect plasma renin concentrations secondary to vascular mediated mechanisms,[33] the results in the aforementioned study[6] were relative to sham-operated control rats.

Only 2 cats, 1 from each kidney storage group, became hypertensive after autograft reperfusion. Two cats received antihypertensive drugs just prior to extubation. Although it is possible this may have skewed the data, there was 1 cat from each group so treated and bias of the data seems unlikely. These drugs were given to prevent potential anastomotic leakage, as these cats were utilized in another study[9] evaluating chronic changes in blood pressure after renal transplantation. Each of these cats was regaining consciousness after anesthesia, and although sufficient analgesia was judged to be present at the time, it is difficult to rule out postoperative pain or dysphoria, fueled by catecholamine release, as potential causes of hypertension. Once treated, hypertension did not recur.

In summary, hemodynamic variables were not significantly different, nor was evidence of increased vasoactive compounds identified following variable ischemic duration or type, respectively. Clinically relevant ischemic episodes did not change renal cortical renin, ACE, or endothelin mRNA concentrations. Reperfusion of allografts may be necessary to observe changes in renin or endothelin. Cold ischemia times up to 3 hours do not appear relevant in inducing hypertension or alterations in the renin-angiotensin system in clinically normal cats receiving a renal autograph.

a. Model TA11PA-C40, 10 cm, Data Sciences International (DSI), Saint Paul, Minn.
b. Model RMC-1, DSI, Saint Paul, Minn.
c. Model APR-1, DSI, Saint Paul, Minn.
d. Data Exchange Matrix, DSI, Saint Paul, Minn.
e. Dataquest A.R.T., version 2.1, DSI, Saint Paul, Minn.
f. RNAlater, Applied Biosystems, Foster City, Calif.
g. RNeasy Kit, Qiagen, Valencia, Calif.
h. High Capacity cDNA Archive Kit, Applied Biosystems, Foster City, Calif.
i. Taqman, SuperArray Bioscience Corp, Frederick, Md.
j. GenBank [database online]. Bethesda, Md: National Institute of Health, 2008. Available at: www.ncbi.nlm.nih.gov/Genbank. Accessed Jul 17, 2008.
k. Primer Express 2.0, Applied Biosystems, Foster City, Calif.
l. SensoLyte Renin Assay Kit 520, donated by AnaSpec, San Jose, Calif.
m. 5-FAM-Pro-Leu-OH, AnaSpec Inc, San Jose, Calif.
n. Fetal Bovine Serum, Atlanta Biologicals, Norcross, Ga.
o. Human renin, recombinant, AnaSpec Inc, San Jose, Calif.
p. Graphpad Prism for Windows, version 5.00, GraphPad Software, San Diego, Calif.

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Appendix

Criteria used to assign a discomfort score to cats after telemetric catheter placement and renal autotransplantation and contralateral nephrectomy.

Observation	Score	Criterion
Comfort and position	0	Asleep or calm and relaxed
	1	Awake; interested in surroundings
	2	Mild agitation or signs of depression; uninterested in surroundings; hunched body posture
	3	Moderate agitation or restless; appears to be uncomfortable; hunched body posture
	4	Extremely agitated or thrashing
Appearance	0	Apparently normal
	1	Mild changes (eyes partially closed)
	2	Moderate changes (eyes sunken or glazed)
	3	Severe changes (eyes pale and pupils dilated; abnormal facial expression; guarding or hunched body posture; limbs in abnormal position)
Behavior (unprovoked)	0	Too sedate to evaluate
	1	Apparently normal; grooming
	2	Minor changes; grooming
	3	Moderately abnormal (less mobile or alert than normal and unaware of surroundings or very restless)
	4	Markedly abnormal (very restless; vocalization or grunting; self-mutilation)
Interactive	0	Too sedate to evaluate
	1	Apparently normal
	2	Pulls away when surgical site is touched; mobile; looking at wound
	3	Vocalizes when surgical wound is touched; reluctant to move but will if coaxed
	4	Violent reaction to touching of surgical site; snapping, hissing, or growling when approached; will not move even when coaxed

Cats were assigned a discomfort score by summing the score in each observation category; if any cat was assigned a score > 8, supplemental analgesia was administered.

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