ONLINE SUPPLEMENT

REINFORCING FEEDBACK LOOP OF RENAL CYCLIC GMP AND INTERSTITIAL HYDROSTATIC PRESSURE IN PRESSURE-NATRIURESIS

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General Methods

Animal Preparation

Animals were anesthetized with either ketamine (60 mg/kg) and xylazine (4 mg/kg) [Protocols 1,2] or pentobarbital (5 mg/100 g body weight) [Protocols 3,4] via an intraperitoneal (IP) injection. Following anesthesia a tracheostomy was performed using polyethylene tubing (PE-240) to assist respiration. Direct cannulation of the right internal jugular vein using PE-10 provided intravenous access through which 5% dextrose in water (D_5W) alone was infused at 20 µL/min. D_5W with inulin and lithium chloride was infused at 20 µL/min for studies involving the measurement of GFR. Cannulation of the right carotid artery using PE-50 provided arterial access for blood pressure (BP) measurements.

For one-kidney models [Protocols 1-3] the right kidney was removed following a midline laparotomy and the remaining ureter was cannulated (PE-10) to collect urine for the quantification of urine sodium (Na^+) excretion ($U_{Na}V$). For the two-kidney model [Protocol 4], both ureters were cannulated individually to collect urine for the quantification of $U_{Na}V$. The right kidney served as the control kidney and received renal interstitial (RI) infusions of vehicle (V) D_5W while the left kidney served as the experimental kidney and received RI infusions of pharmacologic agents. All experiments were performed at similar times each day to prevent diurnal variation in BP measurements.

Kidney weights were measured in a group of 10 animals in order to normalize all data per gram of kidney weight. The mean weight per kidney was 0.978 ± 0.031 grams. We have approximated this to be 1 gram.

Blood Pressure Measurements

Mean arterial pressure (MAP) was measured through the carotid artery using a digital BP analyzer (Digi-Med). Pressures were recorded every 5 minutes and averaged for all periods in the study. MAP served as a surrogate for renal perfusion pressure (RPP) in our experiments.

Hematocrit Measurements

Hematocrit values were measured in a group of 12 animals in order to evaluate hydration status both before and after surgery. Prior to anesthesia (pentobarbital) and again 3 hours after surgery, whole arterial blood was obtained from the animal via tail stick, and spun for 3 minutes by centrifuge (Readacrit, BD Diagnostic Systems). Hematocrit was determined using a Spirocrit microhematocrit capillary tube reader (Monoject Scientific). The mean hematocrit prior to anesthesia was 46.67 ± 0.85%, and after surgery was 46.17 ± 1.30% (*P*=NS).

Pharmacologic Agents

Cyclic guanosine 3’5’-monophosphate (cGMP; BioLog) was used in this study to induce natriuresis. Probenecid (PB; Sigma) was employed to block organic anion transport and inhibit cGMP from leaving renal proximal tubule (RPT) cells. 1-*H*-[1,2,4] oxadiazolo-[4,2-α] quinoxalin-1-one (ODQ; Alexis Biochemicals) was

used to inhibit the cGMP-producing enzyme soluble guanylyl cyclase (sGC). 2% albumin (Sigma) constituted in 0.9% saline was used to increase RIHP artificially. Inulin from dahlia tubers (Sigma) was used to quantify glomerular filtration rate (GFR) and fractional excretion of Na^+ (FE_{Na}). Lithium chloride (Sigma) was used to measure fractional excretion of lithium (Li^+) (FE_{Li}).

SUPPLEMENTAL FIGURES

FIGURE S1

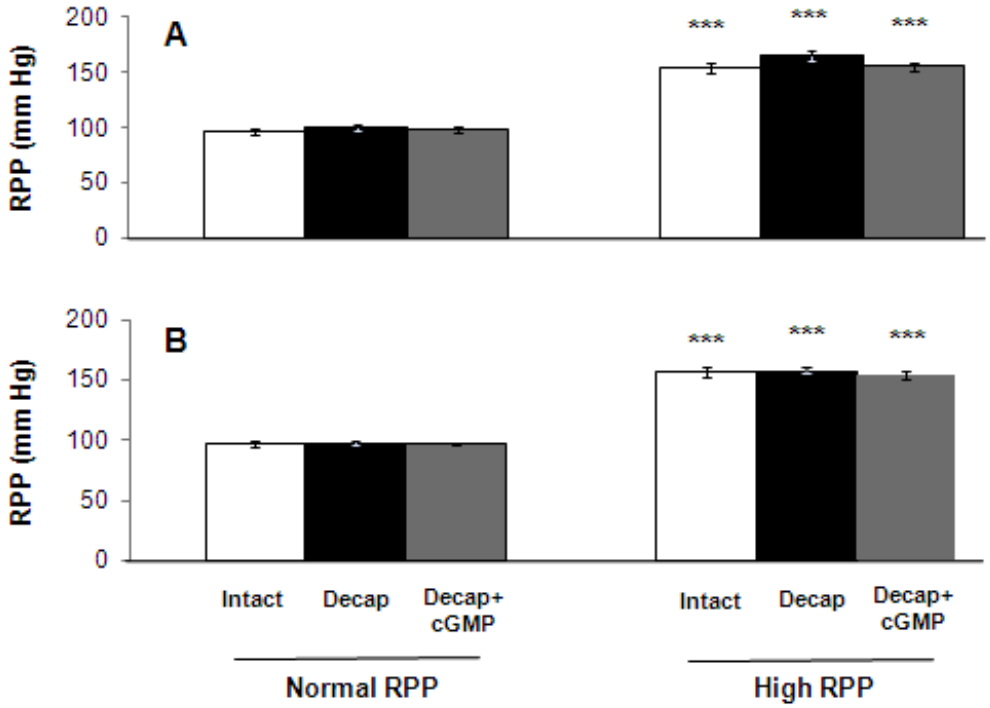

Figure S1. **A**, Renal perfusion pressure (RPP) in uninephrectomized, anesthetized rats with an intact renal capsule (white bars, n=7), after partial decapsulation (Decap) (black bars, n=6), or after partial decapsulation with RI cortical infusion of cGMP during the experimental period (gray bars, n=7). **B**, RPP in uninephrectomized, anesthetized rats with an intact capsule (white bars, n=8), after partial decapsulation (black bars, n=7), or after partial decapsulation with RI cortical infusion of cGMP during the experimental period (gray bars, n=7). Data are shown as mean ± SE. $^{***}P<0.001$ vs own control.

FIGURE S2

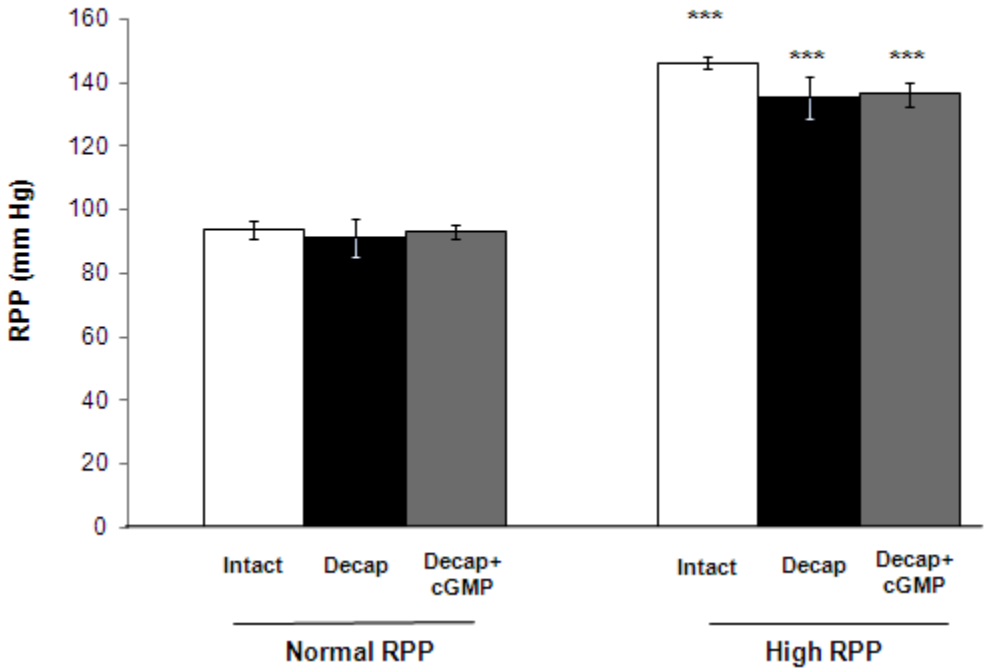

Figure S2. Renal perfusion pressure (RPP) in uninephrectomized, anesthetized rats with an intact renal capsule (white bars, n=8), after partial decapsulation (Decap) (black bars, n=8), or after partial decapsulation with RI cortical infusion of cGMP during the experimental period (gray bars, n=8). Data are shown as mean ± SE. $^{***}P<0.001$ vs own control.

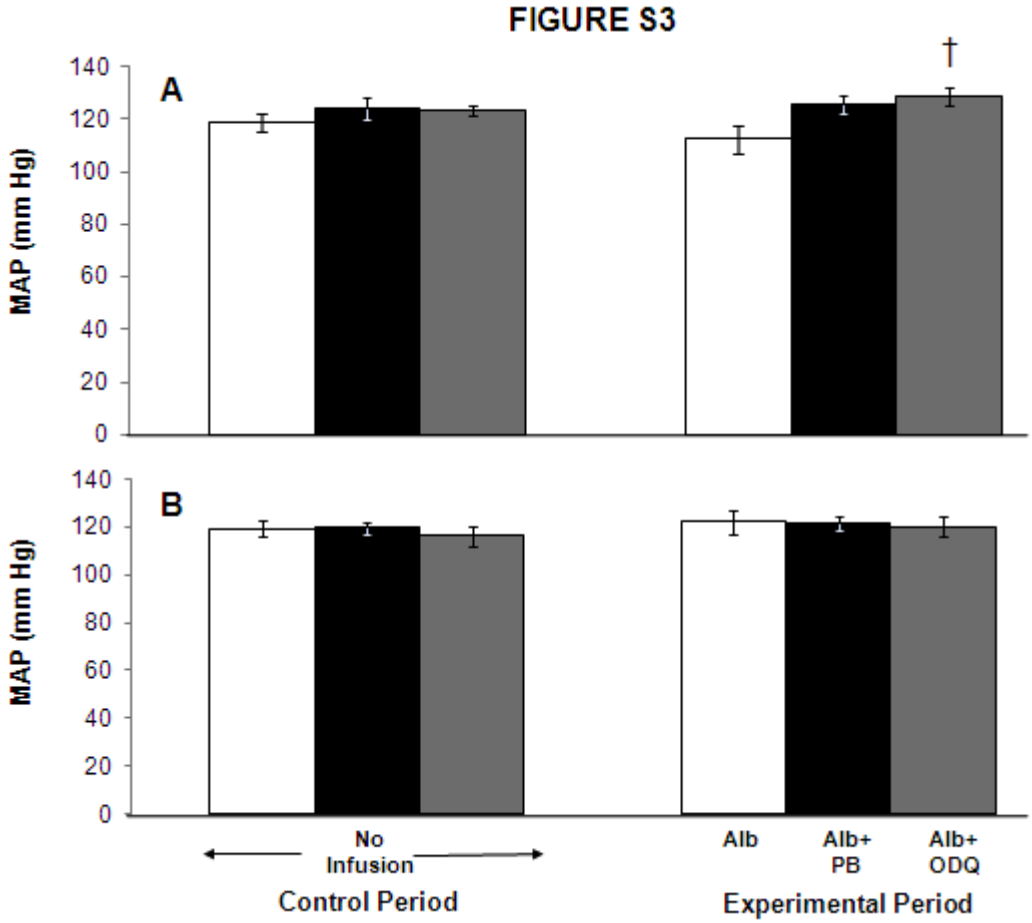

Figure S3. **A**, Mean arterial pressure (MAP) in uninephrectomized, anesthetized rats during control and experimental periods in response to RI bolus of 2% albumin (Alb) during the experimental period (white bars, n=5), RI bolus of albumin followed by RI infusion of probenecid (PB) (black bars, n=7), and RI bolus of albumin followed by RI infusion of ODQ (gray, n=8). **B**, Mean arterial pressure (MAP) in uninephrectomized, anesthetized rats during control and experimental periods in response to RI bolus of 2% albumin (Alb) during the experimental period (white bars, n=10), RI bolus of albumin followed by RI infusion of probenecid (PB) (black bars, n=7), and RI bolus of albumin followed by RI infusion of ODQ (gray bars, n=13). Data are shown as mean ± SE. $^{\dagger}P<0.05$ vs albumin bolus alone (ANOVA with Dunnett's).

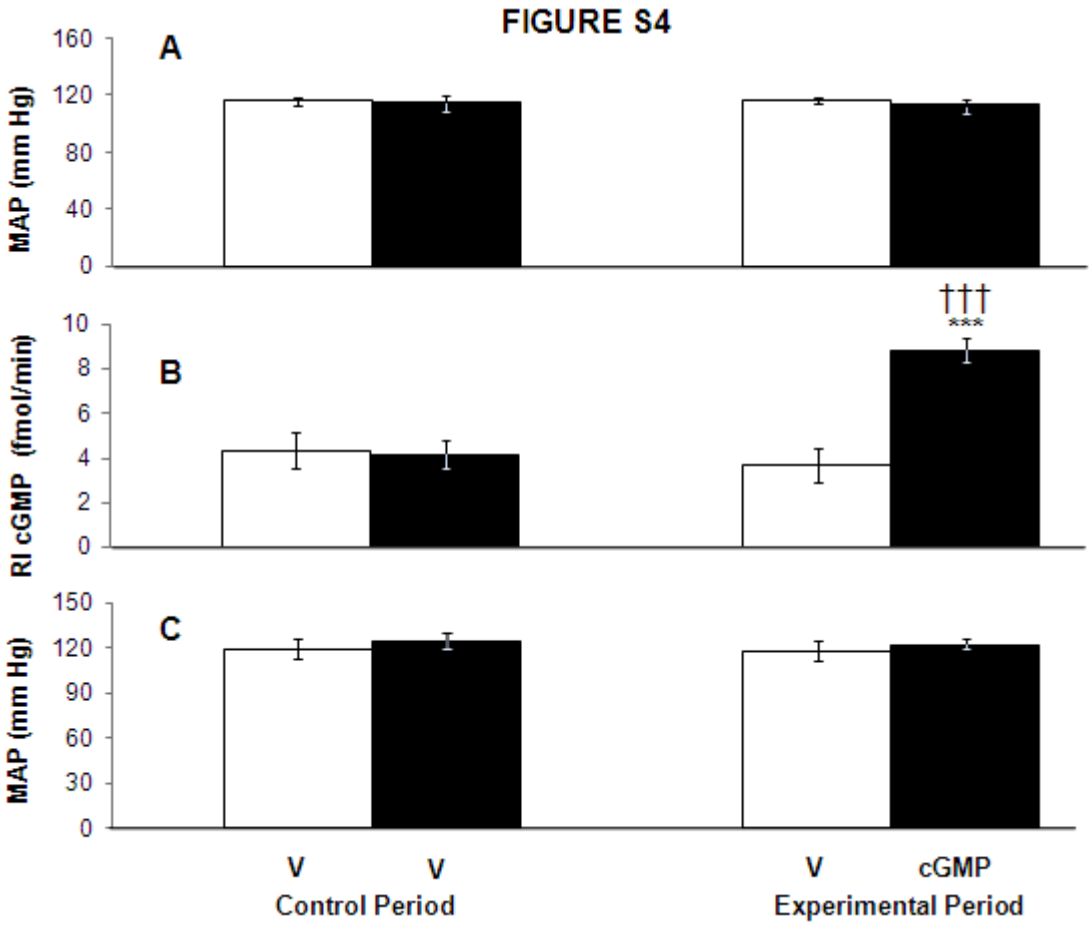

Figure S4. **A**, Mean arterial pressure (MAP) in uninephrectomized, anesthetized rats during control and experimental periods in response to renal interstitial (RI) cortical infusion of D_5W vehicle (V) during both periods (time control, white bars, n=8) or cGMP during the experimental period (black bars, n=10). **B**, RI cGMP in uninephrectomized, anesthetized rats during control and experimental periods in response to RI cortical infusion of D_5W vehicle (V) during both periods (time control, white bars, n=6) or cGMP during the experimental period (black bars, n=6). **C**, MAP in uninephrectomized, anesthetized rats during control and experimental periods in response to RI infusion of D_5W vehicle (V) during both periods (time control, white bars, n=6) or cGMP during the experimental period (black bars, n=6). Data are shown as mean ± SE. $^{***}P<0.001$ vs own control; $^{†††}P<0.001$ vs time control (Two-sample Student's *t* test).

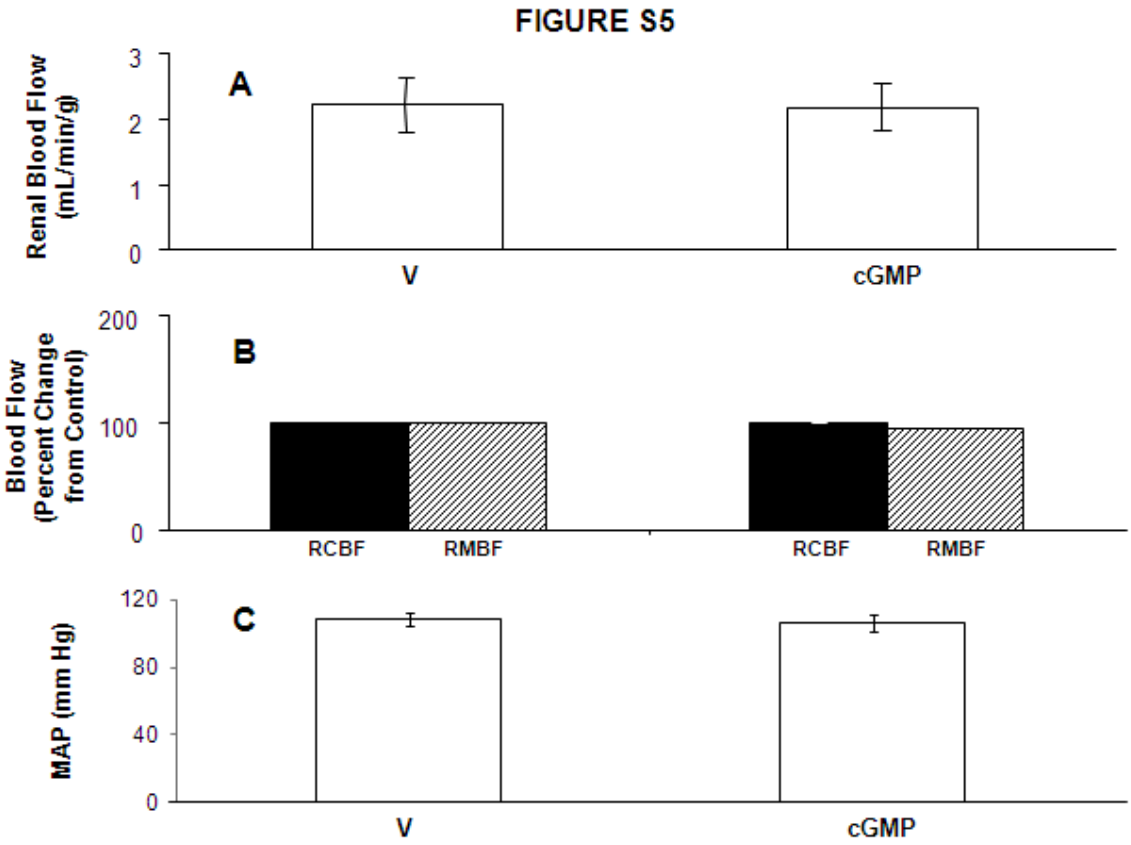

Figure S5. **A**, Renal blood flow (RBF) in uninephrectomized rats (n=7) in response to either D_5W vehicle (V) or cGMP via renal cortical interstitial infusion. Results are reported per gram (g) kidney weight. **B**, Renal cortical blood flow (RCBF, solid bars) and renal medullary blood flow (RMBF, hatched bars) in uninephrectomized anesthetized rats (n=7) in response to either V or cGMP as per A. **C**, Mean arterial pressure (MAP) in uninephrectomized, anesthetized rats (n=7) in response to either V or cGMP as per A. Data are shown as mean ± SE, or as percent change from control, ± SE.

FIGURE S6

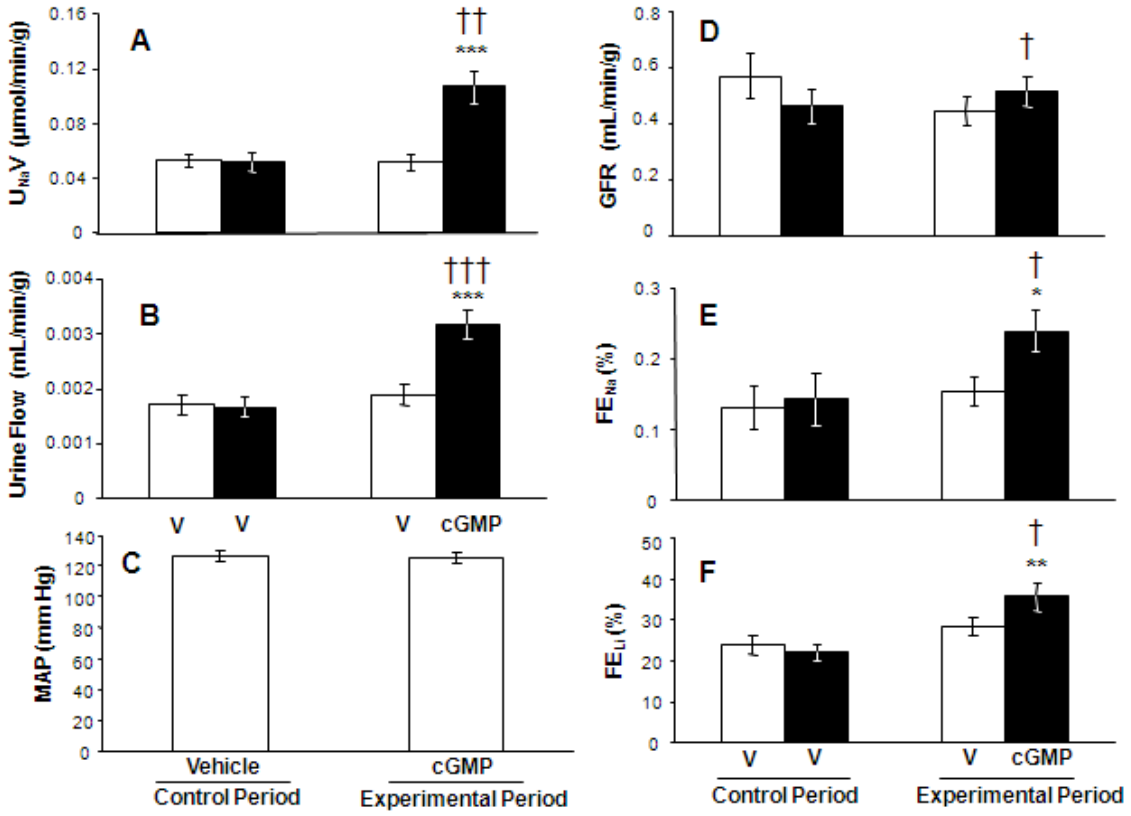

Figure S6. **A**, Urine Na^+ excretion ($U_{Na}V$) in two-kidney model, anesthetized rats (n=15) during control and experimental periods in response to renal interstitial (RI) cortical infusion of D_5W vehicle (V) during both periods (time control, right kidney, white bars) or cGMP during the experimental period (left kidney, black bars). Results are reported per gram (g) kidney weight. **B**, urine flow rate in response to RI infusions as for A (n=14). Results are reported per gram (g) kidney weight. **C**, Mean arterial pressure (MAP) in response to RI infusions as for A (n=15). **D**, Glomerular filtration rate (GFR) in response to RI infusions as for A (n=15). Results are reported per gram (g) kidney weight. **E**, Fractional excretion of Na^+ (FE_{Na}) in response to RI infusions as for A (n=14). **F**, Fractional excretion of Li^+ (FE_{Li}) in response to RI infusions as for A (n=15). Data are shown as mean ± SE. ***P<0.001, **P<0.01, *P<0.05 vs own control; $^{\dagger\dagger\dagger}P$<0.001, $^{\dagger\dagger}P$<0.01, $^{\dagger}P$<0.05 vs time control (paired Student's t test).