Protein Kinase C and $G_{i/o}$ Proteins Are Involved in Adenosine- and Ischemic Preconditioning–Mediated Renal Protection

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Abstract. Renal ischemic reperfusion (IR) injury is a significant clinical problem in anesthesia and surgery. Recently, it was demonstrated that both renal ischemic preconditioning (IPC) and systemic adenosine pretreatment protect against renal IR injury. In cardiac IPC, pertussis toxin-sensitive G-proteins ($i.e., G_{i/o}$), protein kinase C (PKC), and ATP-sensitive potassium ($K^{+}_{ATP}$) channels are implicated in this protective signaling pathway. The aim of this study was to elucidate the signaling pathways that are responsible for renal protection mediated by both IPC and adenosine pretreatment. In addition, because $A_1$ adenosine receptor antagonist failed to block renal IPC, whether activation of bradykinin, muscarinic, or opioid receptors can mimic renal IPC was tested because these receptors have been implicated in cardiac IPC. Rats were acutely pretreated with chelerythrine or glibenclamide, selective blockers of PKC and $K^{+}_{ATP}$ channels, respectively, before IPC or adenosine pretreatment. Some rats were pretreated with pinacidil ($K^{+}_{ATP}$ channel opener), bradykinin, methacholine, or morphine before renal ischemia. Twenty-four h later, plasma creatinine was measured. Separate groups of rats received pertussis toxin intraperitoneally 48 h before being subjected to the above protective protocols. IPC and adenosine pretreatment protected against renal IR injury. Pretreatment with pertussis toxin and chelerythrine abolished the protective effects of both renal IPC and adenosine. However, glibenclamide pretreatment had no effect on either renal IPC or adenosine-induced renal protection, indicating no apparent role for $K^{+}_{ATP}$ channels. Moreover, pinacidil, bradykinin, methacholine, and morphine failed to protect renal function. Therefore, the conclusion is that cellular signal transduction pathways of renal IPC and adenosine pretreatment in vivo involve $G_{i/o}$ proteins and PKC but not $K^{+}_{ATP}$ channels. Unlike cardiac IPC, bradykinin, muscarinic, and opioid receptors do not mediate renal IPC.

Renal dysfunction secondary to ischemic reperfusion (IR) injury contributes to frequent and grave clinical morbidity in aortoarterial surgery and anesthesia (1,2). Postoperative acute renal failure implies a poor clinical prognosis and is frequently associated with many other life-threatening complications, including sepsis and multiorgan failure (1–3). In high-risk patients undergoing major vascular surgery and anesthesia, the mortality and morbidity rate from perioperative acute renal failure has changed little during the past 30 yr; the incidence of renal dysfunction after aortoarterial surgery is reported to be as high as 50% (3).

Murry (4), in 1986, first reported the protective effects of “ischemic preconditioning (IPC)” against IR injury in cardiac muscle by showing that multiple brief ischemic periods before a prolonged ischemic period lessened myocardial dysfunction and infarction size after the reperfusion period. We recently demonstrated that IPC protects renal function and morphology in rats after 45 min of ischemia and 24 h of reperfusion (5).

Extensive studies of cardiac IPC have implicated pre-ischemic activation of adenosine receptors (AR), specifically, $A_1$ AR, as a predominate mechanism that mediates protection (6,7). In addition, it is hypothesized in cardiac models of IPC that stimulation of $A_1$ AR results in activation and opening of ATP-sensitive potassium ($K^{+}_{ATP}$) channels involved (6,10). In models of cardiac protection, PKC and $K^{+}_{ATP}$ channel activation are thought to be coupled to cell surface $A_1$ AR via pertussis toxin-sensitive G-proteins ($i.e., G_{i/o}$ (11,12)). Therefore, we hypothesized that $G_{i/o}$, PKC, and $K^{+}_{ATP}$ channels are intermediate signaling proteins involved in adenosine- and IPC-mediated renal protection from IR injury as has been demonstrated in the heart.

We recently demonstrated that systemic adenosine pretreatment protects renal function via $A_1$ AR activation and mimics renal IPC (5). However, unlike most models of cardiac preconditioning, renal IPC was not blocked by an $A_1$ AR antagonist. This suggests either that IPC- and adenosine-mediated...
protection follow completely different cellular signaling pathways with a common physiologic end point or that multiple endogenous agonists with common intermediate signaling pathways are involved in renal IPC. Evidence from cardiac preconditioning suggests that endogenously released agonists other than adenosine, e.g., bradykinin, acetylcholine, and opioid agonists, can also mimic IPC (13–17).

The distal portion (S3 segment) of the proximal tubule located in the outer medulla of the kidney is the primary site of injury in renal ischemia and reperfusion (18,19) because of its marginal oxygenation under normal physiologic conditions coupled with high basal metabolic demand (19–21). These renal tubular cells express the A1 AR (22) as well as the bradykinin (23,24), muscarinic (25,26), and opioid (27,28) receptors. Therefore, the second hypothesis of the current study was that bradykinin, muscarinic, or opioid receptors may mimic the protection induced by A1 AR activation.

Materials and Methods

General Surgical Preparation

All protocols were approved by the Institutional Animal Care and Use Committee of Columbia University. Surgical procedures have been previously described (5). Briefly, adult male Wistar rats were anesthetized with pentobarbital intraperitoneally (45 mg/kg body wt or to effect) and allowed to breathe room air spontaneously. After 500 U of heparin (intraperitoneally) and maintaining the body temperature at 37°C, the right femoral artery and vein were cannulated with heparinized (10 U/ml) polyethylene tubing (PE-50). After a 10-min stabilization period, a midline laparotomy was performed. A right nephrectomy was performed, and the left renal artery and vein were isolated.

IPC and Adenosine Pretreatment

For IPC and adenosine pretreatment protocols, rats were subjected to the following protocols after right nephrectomy as described previously (5): (1) control group (SHAM), isolation of left renal artery and vein only; (2) ischemia-reperfusion group (IR), 45 min of left renal ischemia followed by reperfusion; (3) ischemic preconditioning group (IPC), four cycles of 8 min of left renal ischemia separated by 5 min of reperfusion periods before 45 min of left renal ischemia followed by reperfusion; and (4) adenosine pretreatment group (ADO), adenosine (1.75 mg/kg per min × 10 min, intravenously) 2 min before 45 min of left renal ischemia followed by reperfusion. Twenty-four h later, animals were killed and serum Cr levels were measured.

Role of PKC in IPC- and Adenosine-Mediated Renal Protection

To determine the potential role of PKC in renal IPC- or adenosine-induced renal protection, we subjected the rats to the following protocols after right nephrectomy: (1) PKC antagonist (chelerythrine) controls (Che+ Sham), chelerythrine (5 mg/kg, intraperitoneally) 15 min before sham operations; (2) chelerythrine and ischemia-reperfusion (Che+IR), chelerythrine 15 min before being subjected to 45 min of left renal ischemia followed by reperfusion; (3) chelerythrine before adenosine (Che+ADO), chelerythrine 15 min before 10 min of adenosine pretreatment followed by 45 min of left renal ischemia followed by reperfusion; and (4) chelerythrine before IPC (Che+IPC), chelerythrine 15 min before IPC treatment followed by 45 min of left renal ischemia followed by reperfusion. Twenty-four h later, animals were killed and serum Cr levels were measured.

Role of K+ ATP Channels in IPC- and Adenosine-Mediated Renal Protection

To determine the potential role of K+ ATP channels in renal IPC- or adenosine-induced renal protection, we subjected the rats to the following protocols after right nephrectomy: (1) high dose K+ ATP channel antagonist (glibenclamide) controls (H-Glib+Sham), glibenclamide (6 mg/kg intravenously) 30 min before sham operations; (2) high dose glibenclamide and ischemia-reperfusion (H-Glib+IR), glibenclamide (6 mg/kg intravenously) 30 min before 45 min of left renal ischemia followed by reperfusion; (3) low dose glibenclamide before adenosine (L-Glib+ADO), glibenclamide (1 mg/kg intravenously) 30 min before 10 min of adenosine pretreatment followed by 45 min of left renal ischemia followed by reperfusion; (4) high dose glibenclamide before adenosine (H-Glib+ADO), glibenclamide (6 mg/kg intravenously) 30 min before 10 min of adenosine pretreatment followed by 45 min of left renal ischemia followed by reperfusion; (5) high dose glibenclamide before IPC (H-Glib+IPC), glibenclamide (6 mg/kg intravenously) 30 min before IPC treatment followed by 45 min of left renal ischemia followed by reperfusion; and (6) pinacidil pretreatment group, pinacidil, a K+ ATP channel opener, (100 μg/kg per min × 10 min intravenously) 2 min before 45 min of left renal ischemia followed by reperfusion. Twenty-four h later, animals were killed and serum Cr levels were measured.

Potential Roles of Muscarinic, Bradykinin, and Opioid Receptors in Renal Protection

To determine whether other agonists with similar signaling pathways in renal cells mimic renal protection afforded by adenosine or IPC, methacholine (a muscarinic agonist), bradykinin, or morphine was given to rats before ischemia and reperfusion. Rats were divided to the following groups after right nephrectomy: (1) methacholine pretreatment group (MCh), methacholine (2 mg/kg, intraperitoneally) 15 min before 45 min of left renal ischemia followed by reperfusion; (2) bradykinin pretreatment group (BK), bradykinin (500 μg/kg per min × 10 min intravenously) terminating 2 min before 45 min of left renal ischemia followed by reperfusion; and (3) morphine pretreatment group (Morph), morphine (5 mg/kg intraperitoneally) 15 min before 45 min of left renal ischemia followed by reperfusion. Twenty-four h later, animals were killed and serum Cr levels were measured. The doses of chelerythrine, glibenclamide, methacholine, bradykinin, and morphine were selected based on previous in vivo studies (15,28–31).

Role of Pertussis Toxin–Sensitive G-Proteins in Renal Protection

To determine the potential role of pertussis toxin–sensitive G-proteins in renal IPC- and adenosine-induced renal protection, we initially pretreated rats for 48 h with pertussis toxin 25 μg/kg intraperitoneally. Pilot studies demonstrated that all of the rats that were pretreated intraperitoneally with 25 μg/kg pertussis toxin and subjected to 45 min of renal ischemia died within 8 h during the reperfusion period with evidence of elevated body temperature and distended fluid-filled small bowel. Therefore, three separate and independent changes in the protocol were made in an attempt to enhance survival after pertussis toxin treatment and ischemia-reperfusion: (1) The dose of pertussis toxin was reduced to 10 μg/kg intraperitoneally; (2) the reperfusion period was shortened to 6 h in some rats, and (3) the ischemic period was shortened from 45 to 30 min. Despite lowering the pertussis toxin dose to 10 μg/kg, none of the rats...
survived more than 10 h after 45 min of renal ischemia. The Cr measured at 6 h of reperfusion indicated that pertussis toxin pretreatment blocked IPC- and adenosine-mediated renal protection (see the Results section). In an attempt to prolong the survival after pertussis toxin (10 μg/kg intraperitoneally) and renal ischemia, the ischemic time interval was reduced to 30 min, resulting in a greater that 60% survival during 24 h of reperfusion. Subsequently, the following protocols were performed: (1) ischemia-reperfusion group with modified ischemia protocol (IR30), 30 min of left renal ischemia and reperfusion; (2) IPC group with modified ischemia protocol (IPC30), four cycles of 8 min of left renal ischemia separated by 5 min of reperfusion periods before 30 min of left renal ischemia followed by reperfusion; (3) adenosine pretreatment group with modified ischemia protocol (ADO30), adenosine (1.75 mg/kg per min × 10 min intravenously) until 2 min before 30 min of left renal ischemia followed by reperfusion; (4) pertussis controls (PTX+SHAM), pertussis toxin (10 μg/kg intraperitoneally) 48 h before the sham operation; (5) pertussis toxin and ischemia-reperfusion group (PTX+IR30), pertussis toxin (10 μg/kg intraperitoneally) 48 h before 30 min of left renal ischemia followed by reperfusion; (6) pertussis toxin before IPC group (PTX+IPC30), pertussis toxin (10 μg/kg intraperitoneally) 48 h before being subjected to four cycles of 8 min of left renal ischemia separated by 5 min of reperfusion periods before 30 min of left renal ischemia followed by reperfusion; (7) pertussis toxin before adenosine group (PTX+ADO30), pertussis toxin (10 μg/kg intraperitoneally) 48 h before receiving an systemic intravenous infusion of adenosine (1.75 mg/kg per min × 10 min) until 2 min before 30 min of left renal ischemia followed by reperfusion.

Effectiveness of \( K^{+}_{\text{ATP}} \) Channel Blockade by Intravenous Glibenclamide In Vivo

To test the effectiveness of glibenclamide in blocking \( K^{+}_{\text{ATP}} \) channels in vivo, we measured hemodynamic and metabolic parameters. Rats received 0.3 mg/kg pinacidil (\( K^{+}_{\text{ATP}} \) channel opener) intravenously while a second group was pretreated with 6 mg/kg of glibenclamide intravenously 30 min before receiving pinacidil (0.3 mg/kg intravenously). Maximal changes in mean arterial BP were recorded for each animal. In addition, blood glucose was measured colorimetrically by Antec Diagnostics (Farmingdale, NY) 60 min after receiving glibenclamide (6 mg/kg intravenously).

Measurement of Cr

Plasma Cr levels were measured spectrophotometrically using a commercially available quantitative colorimetric assay (Sigma, St. Louis, MO).

Materials

Adenosine, pertussis toxin, and methacholine were dissolved in sterile, isotonic saline. All other drugs were dissolved in 50% DMSO. Solutions were made daily. Pentobarbital was purchased from Henry Schein Veterinary Co. (Indianapolis, IN). All other drugs were obtained from Sigma Chemical Company.

Statistical Analyses

A one-way ANOVA was used to compare mean values across multiple treatment groups with a Dunnett post hoc multiple comparison test, e.g., SHAM versus IPC. In all cases, a probability statistic less than 0.05 was taken to indicate significance. All data are expressed throughout the text as mean ± SEM.

Results

Protective Effects of Renal IPC and Systemic Adenosine Pretreatment

Forty-five min of renal ischemia and 24 h of reperfusion (IR) resulted in significant rises in Cr (4.6 ± 0.3 mg/dl \( n = 9 \)) compared with the sham-operated group (Cr = 0.8 ± 0.1 mg/dl \( n = 12 \); \( P < 0.01 \); Figure 1). IPC significantly improved renal function (Cr = 2.5 ± 0.4 mg/dl \( n = 9 \); \( P < 0.05 \)) after 45 min of renal ischemia and 24 h of reperfusion compared with animals that were subjected to IR injury alone (Figure 1). Systemic adenosine (ADO) pretreatment also resulted in significant improvements in renal function (Cr = 1.7 ± 0.4 mg/dl \( n = 12 \); \( P < 0.01 \); Figure 1) compared with animals that were subjected to IR injury alone.

Roles of PKC and \( K^{+}_{\text{ATP}} \) Channels in Renal IPC- and Adenosine-Induced Renal Protection

Chelerythrine (Che, 5 mg/kg, intraperitoneally), a PKC antagonist, given 15 min before IPC or adenosine infusion abolished the renal protection induced by IPC (Cr = 4.1 ± 0.4 mg/dl \( n = 9 \)) or adenosine (Cr = 4.4 ± 0.5 mg/dl \( n = 10 \); Figure 1). Chelerythrine itself had no effect on renal function of sham-operated rats (Cr = 0.7 ± 0.1 mg/dl \( n = 4 \)) or of rats that underwent 45 min of renal ischemia and 24 h of reperfusion (Cr = 4.4 ± 0.8 mg/dl \( n = 3 \)).

In contrast to the effects of chelerythrine, both the low (L-Glib, 1 mg/kg) and high (H-Glib, 6 mg/kg) doses of glibenclamide, a selective antagonist for \( K^{+}_{\text{ATP}} \) channels, given 30 min before adenosine failed to block the renal protection by systemic adenosine pretreatment (low dose: Cr = 1.8 ± 0.5 mg/dl \( n = 4 \); high dose: Cr = 1.7 ± 0.4 mg/dl \( n = 6 \); Figure 2). High-dose glibenclamide also failed to block the renal protective effects of renal ischemic preconditioning (IPC) on Cr compared with sham-operated rats (Che+IR, n = 3).
Glibenclamide (6 mg/kg intravenously) given alone had no effect on renal function of sham-operated rats (Cr = 1.1 ± 0.1 mg/dl [n = 3]) or of rats that were subjected to ischemia and reperfusion (Cr = 4.0 ± 0.2 mg/dl [n = 4]). Moreover, pretreatment with pinacidil, a K\textsuperscript{+} channel opener, failed to protect renal function (Cr = 4.0 ± 0.3 mg/dl [n = 3]).

**Effectiveness of K\textsuperscript{+} \textsubscript{ATP} Channel Antagonism by Glibenclamide In Vivo**

Activation of vascular K\textsuperscript{+} \textsubscript{ATP} channels leads to hypotension in vivo. Figure 3 shows the hypotensive response (maximum drop in mean arterial BP = 78 ± 16 mmHg [n = 3]) to pinacidil (0.3 mg/kg intravenously), a K\textsuperscript{+} \textsubscript{ATP} channel opener. Pretreatment with glibenclamide (6 mg/kg intravenously) 30 min before pinacidil bolus significantly attenuated the hypotension (maximum drop in mean arterial BP = 13 ± 6 mmHg [n = 3]; P < 0.05), indicating an effective in vivo blockade of vascular K\textsuperscript{+} \textsubscript{ATP} channels.

We also measured blood glucose levels before and 60 min after glibenclamide (6 mg/kg intravenously). Blockade of pancreatic K\textsuperscript{+} \textsubscript{ATP} channels results in an increased release of insulin and subsequent fall in blood glucose. Glibenclamide significantly decreased the blood glucose level from 224 ± 40 mg/dl [n = 3] to 31 ± 11 mg/dl 60 min after injection (P < 0.01). Control rats that were not treated with glibenclamide had significantly higher blood glucose levels (152 ± 15 mg/dl [n = 3]) 60 min after initiation of the surgical procedure (preoperative blood glucose = 208 ± 12 mg/dl [n = 3]). Taken together, these physiologic effects of glibenclamide suggest an effective blockade of K\textsuperscript{+} \textsubscript{ATP} channels in the present study.

**Pertussis Toxin Abolished Protective Effects of Renal IPC and Adenosine Pretreatment**

The bradycardic responses to adenosine and R-phenylisopropyladenosine (R-PIA) are known to be mediated via activation of A\textsubscript{1} AR that couple to G\textsubscript{i/o} (32,33). Forty-eight h of either 10 or 25 μg/kg pertussis toxin treatment abolished the bradycardic effects of R-PIA and adenosine (data not shown), indicating effective in vivo blockade of G\textsubscript{i/o} by our pertussis toxin pretreatment regimen.

Pertussis toxin pretreatment alone had no effect on animals’ hemodynamic profile, apparent well-being, or appearance. Moreover, pertussis toxin–treated rats that underwent sham operations had similar renal function (Cr = 1.1 ± 0.1 mg/dl [n = 2]) compared with the sham-operated controls (Cr = 0.8 ± 0.1 mg/dl [n = 12]). However, none of the pertussis toxin–treated animals (either 10 or 25 μg/kg) survived more than 8 h after being subjected to 45 min of renal ischemia. Therefore, in the initial group of experiments, the reperfusion period was shortened to 6 h in both the pertussis toxin–treated and control groups. Forty-five min of renal ischemia and 6 h of reperfusion resulted in significant rises in Cr (2.7 ± 0.2 mg/dl [n = 6]), although these rises as expected were much less than those of rats that were subjected to 45 min of renal ischemia and 24 h of reperfusion (Cr = 4.6 ± 0.3 mg/dl [n = 9]). IPC (Cr = 1.4 ± 0.1 mg/dl [n = 6]) and R-PIA (A\textsubscript{1} AR agonist) pretreatment (Cr = 1.9 ± 0.1 mg/dl [n = 6]) also protected renal function after 45 min of renal ischemia and the shorter reperfusion period of 6 h. However, pretreatment with pertussis toxin (25 μg/kg intraperitoneally) 48 h before renal IPC (Cr = 3.0 ± 0.4 mg/dl [n = 6]) and A\textsubscript{1} AR agonist (Cr = 2.7 ± 0.4 mg/dl, [n = 6]) abolished renal protection by either A\textsubscript{1} AR activation or IPC.

In an attempt to enhance survival beyond 24 h, both the dose of pertussis toxin and the duration of renal ischemia were reduced in the next series of experiments, which resulted in more than 60% of the animals surviving 24 h of reperfusion. Thirty min of renal ischemia and 24 h of reperfusion resulted in significant rises in Cr (4.1 ± 0.2 mg/dl [n = 3]; Figure 4). IPC (Cr = 1.5 ± 0.2 mg/dl [n = 3]) and adenosine pretreat-
ment (Cr = 1.3 ± 0.1 mg/dl [n = 3]) protected renal function in these rats (Figure 4). The rats that were pretreated for 48 h with 10 μg/kg pertussis toxin intraperitoneally and then subjected to 30 min of renal ischemia and 24 h of reperfusion also exhibited significant impairments of renal function (Cr = 4.4 ± 0.5 mg/dl [n = 4]). However, pretreatment with 10 μg/kg pertussis toxin 48 h before renal IPC (Cr = 5.0 ± 0.3 mg/dl [n = 3]) or systemic adenosine (Cr = 5.3 ± 0.5 [n = 3]) abolished their renal protective effects (Figure 4). These data suggest that pertussis toxin–sensitive G-proteins are signaling intermediates in both adenosine- and IPC-mediated protection of renal IR injury.

Methacholine, Bradykinin, and Morphine Failed to Protect Renal Function

Intravenous bradykinin at 500 μg/kg per min caused a similar degree of hypotension (systolic BP, approximately 60 to 70 mmHg) as observed with intravenous adenosine (5). Systemic methacholine caused transient reduction in BP but was associated with markedly increased salivary and lacrimal secretions. Pretreatments with systemic methacholine (Cr = 3.8 ± 0.1 mg/dl [n = 6]), bradykinin (Cr = 4.6 ± 0.1 mg/dl [n = 5]), or morphine (Cr = 4.3 ± 0.2 mg/dl [n = 4]) failed to protect renal function (Figure 5). This is in contrast to protections obtained with either 10 min of systemic adenosine pretreatment or with renal IPC (5).

Discussion

The current in vivo study in the rat kidney demonstrates that renal IPC- and adenosine-mediated renal protection from IR injury involve both pertussis toxin–sensitive G-proteins and PKC. In contrast to the findings in most models of cardiac and skeletal muscle preconditioning (6,10,34), K+_{ATP} channels are not involved in renal protection. In addition, unlike adenosine (6,7) and unlike some models of cardiac preconditioning (13–15,35), pretreatments with bradykinin, methacholine, or morphine, agonists that are known to mimic IPC in cardiac muscle, failed to protect renal function after ischemia and reperfusion. The current study is the first to identify signaling intermediates that are responsible for renal IPC- and adenosine-induced renal protection.

The results of our studies have similarities and differences to IPC studies in the heart. In our previous study (5), we were able to protect renal function with pre-ischemic A1 AR activation and mimic renal IPC. However, we were unable to block the protective effects of renal IPC with an A1 AR antagonist. We hypothesized either that IPC- and adenosine-induced protection in the kidney follows completely different cellular signaling pathways or that multiple endogenous agonists are involved in renal IPC. This second hypothesis led us to test whether activation of other endogenous receptors such as muscarinic, bradykinin, or opioid receptors protected renal function against IR injury. In some studies of cardiac IR injury, other receptors that demonstrate common intracellular signaling intermediates with adenosine, such as bradykinin (14,15), acetylcholine (13), phenylephrine (36), and opioids (35), mimic IPC. That multiple agonists (bradykinin, morphine, acetylcholine, and phenylephrine) are able to induce cardiac preconditioning suggested to us that A1 AR activation per se may not be the only agonist inducing protection but that any agonist that stimulates common second messenger signaling intermediates (e.g., G_{i/o} and PKC), may also protect the heart against IR injury. However, unlike studies in the heart, we did not find that activation of muscarinic, bradykinin, or opioid receptors mimicked renal IPC, suggesting that activation of G_{i/o} alone was not sufficient to mediate renal protection. Alternatively, it is possible that the rat renal cells that are protected by IPC and

![Figure 4](image1.png)  
**Figure 4.** Pretreatment with pertussis toxin blocks adenosine- and IPC-mediated renal protection from IR injury. Comparison of mean Cr measured from sham-operated (Sham, n = 4), 48-h pertussis toxin treatment before sham operation (PTX + Sham, n = 2), ischemic-reperfusion (IR30, n = 3), ischemic-preconditioned (IPC30, n = 3), adenosine-pretreated (ADO30, n = 3), 48-h pertussis toxin treatment before ischemia and reperfusion (PTX + IR30, n = 4), 48-h pertussis toxin treatment before IPC (PTX + IPC30, n = 3), and 48 h pertussis toxin treatment before adenosine (PTX + ADO30, n = 3) animals. For this series of study, all groups underwent 30 min of global renal ischemia and 24 h of reperfusion. *, P < 0.05 versus sham; #, P < 0.05 versus IR.

![Figure 5](image2.png)  
**Figure 5.** Bradykinin, methacholine, or morphine did not mimic adenosine-mediated renal protection from IR injury. Comparison of mean Cr measured from sham-operated (Sham, n = 12), IR (IR, n = 9), adenosine-pretreated (ADO, n = 9), bradykinin-pretreated (BK, n = 6), methacholine-pretreated (MCh, n = 6), and morphine-pretreated (Morph, n = 4) animals before ischemia and reperfusion. *, P < 0.05 versus sham; #, P < 0.05 versus IR.
adenosine pretreatment may not express muscarinic, bradykinin, or opioid receptors that couple to G\textsubscript{i/o}.

The current study demonstrated that PKC plays a role in renal IPC- and adenosine-induced renal protection in vivo. We used chelerythrine, a highly selective and specific PKC antagonist that inhibits the PKC catalytic domain (K\textsubscript{i} = 0.7 \mu M (37)), to block the physiologic effects of PKC to determine whether PKC activation is required for renal IPC- and adenosine-induced renal protection. It is accepted that PKC plays a critical role in mediating IPC- and A\textsubscript{1} AR-mediated cardiac protection (8,30). PKC modulates both short- and long-term cellular responses after IR injury, such as ion channel regulation, new protein synthesis, and cellular proliferation. Coupling of A\textsubscript{1} AR to PKC via G\textsubscript{i/o} in renal tubules has been confirmed previously (38–40). Currently, it is not conclusively known which subtypes of PKC are involved in mediating cardiac preconditioning, although evidence exists that the \( \delta \) and/or \( \varepsilon \) isoforms may be involved in the heart (8).

A\textsubscript{1} AR, including those present in the kidney, couple to intracellular effectors via G\textsubscript{i/o} (pertussis toxin–sensitive G-proteins (22,32)). In cardiac IPC from a number of species including the rat, G\textsubscript{i/o} are intermediaries in modulating adenosine’s protective effect (11). Moreover, cardiac preconditioning in vivo is abolished after the pertussis toxin treatment (12,41). Therefore, we hypothesized that by blocking G\textsubscript{i/o} with pertussis toxin, we may prevent the renal protective effects of IPC and adenosine. The doses of pertussis toxin used in this study (25 and 10 \( \mu g/kg \)) were based on previous studies of cardiac IPC in rats (11,12,41). Pertussis toxin treatment for 48 h abolished the protective effects of renal IPC and adenosine pretreatment, supporting a role for G\textsubscript{i/o} proteins as intermediaries in renal IPC- and adenosine-induced renal protection from IR injury. Although we did not measure the degree of adenosine diphosphate ribosylation of G\textsubscript{i/o} in this study, that A\textsubscript{1} AR-mediated bradycardic responses to R-PIA and adenosine were abolished in pertussis toxin–treated rats suggests that A\textsubscript{1} AR-G\textsubscript{i/o} coupling was blocked with pertussis toxin treatment. Endoh et al. (42) also demonstrated that pertussis toxin at doses ranging from 1.25 to 10 \( \mu g/kg \) dose-dependently attenuated the negative inotropic and chronotropic effects of atrial muscarinic receptor activity; the pertussis toxin dose of 10 \( \mu g/kg \) maximally inhibited the receptor-G\textsubscript{i/o} interactions.

That pertussis toxin treatment associated with in vivo renal ischemia led to significant mortality and morbidity is a limitation in our study. None of the rats survived for 24 h after 45 min of global renal ischemia with either 10 or 25 \( \mu g/kg \) of pertussis toxin. We were able to improve the survival rate by reducing the dose of pertussis toxin to 10 \( \mu g/kg \) and the ischemic time to 30 min. With this reduced dose of pertussis toxin and reduced ischemic interval, we enhanced the survival at 24 h of reperfusion and again showed that pertussis toxin pretreatment blocked both adenosine- and IPC-mediated protection from renal IR injury. It is unclear why the combination of pertussis toxin and renal ischemia in vivo is detrimental to rat survival. Sham-operated rats that received 10 to 25 \( \mu g/kg \) of pertussis toxin seemed normal before and after the surgical procedures.

K\textsubscript{ATP} channels are present in various cell types in the kidney, including the proximal tubule, the thick ascending limb of Henle’s loop, and the cortical collecting duct (43). Under physiologic conditions, these channels have a high open probability and function to regulate renal blood flow, reabsorption of electrolytes and solutes, renin release, K\textsuperscript{+} secretion, and diuresis (43–45). In the heart and skeletal muscle, glibenclamide, a selective K\textsubscript{ATP} channel blocker, at doses ranging from 0.3 mg/kg to 3 mg/kg blocked the protective effects of IPC and adenosine (31,34,46). Moreover, the K\textsuperscript{+} ATP channels play a role to protect the brain against IR injury (47). These data suggest that IPC in these tissues may be mediated by the activation of K\textsuperscript{+} ATP channels coupled to A\textsubscript{1} AR and that these channels may serve an endogenous protective role. However, the current study does not support the hypothesis that renal protection by IPC or adenosine pretreatment involves the activation of K\textsuperscript{+} ATP channels. Neither the high (6 mg/kg) nor the low (1 mg/kg) doses of glibenclamide abolished the renal protection afforded by adenosine or IPC. Moreover, pinacidil, a K\textsuperscript{+} ATP channel activator, failed to protect renal function. Our hemodynamic and blood glucose data show that 6 mg/kg glibenclamide intravenously effectively blocked the K\textsuperscript{+} ATP channels in vivo. This finding makes the kidney unique compared with the brain, heart, and skeletal muscle, where K\textsuperscript{+} ATP channels are known to play an important role in tissue protection. The role of K\textsuperscript{+} ATP channels in excitable tissues such as brain, heart, and skeletal muscle seems to be different from the role of K\textsuperscript{+} ATP channels in cells of epithelial origin, e.g., renal tubules, in terms of cytoprotection.

In summary, we extended our in vivo studies in the rat to show that both G\textsubscript{i/o} and PKC are signaling intermediates involved in renal protection induced by either IPC or adenosine pretreatment. Unlike the findings in cardiac models of protection from IR injury, K\textsuperscript{+} ATP channels are not signaling intermediates in renal protection. Moreover, bradykinin, methacholine, or opioid receptor activation does not induce renal protection from IR injury. These studies offer a mechanistic insight into the signaling intermediates that are responsible for adenosine-induced and IPC-induced renal protection from IR injury.

Acknowledgments
This work was funded in part by intramural grant support from the Department of Anesthesiology, College of Physicians and Surgeons of the Columbia University.

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