Role of Adenosine in Renal Protection Induced by a Brief Episode of Ischemic Preconditioning in Rats

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ABSTRACT—The protective effect of a brief episode of ischemic preconditioning was examined at an early phase of ischemic-reperfusion injury in the rat kidney. Rats were subjected to 50 min of left renal artery occlusion followed by 120 min of reperfusion. Ischemic preconditioned rats were subjected to preconditioning with two cycles of 3-min ischemia and 5-min reperfusion (IPC). Ischemic-reperfusion injury led to a low recovery of the glomerular filtration rate (GFR). Overt morphological changes, consisting of blood trapping and tubular collapse, were seen. IPC improved the recovery of GFR and renal morphology. The IPC effect was not blocked by 8-(p-sulfophenyl)-theophylline (SPT), a non-selective adenosine receptor antagonist, by 1,3-dipropyl-8-cyclopentylxanthine (DPCPX), a selective A1-receptor antagonist, or by 3,7-dimethyl-1-propargylxanthine (DMPX), a selective A2-receptor antagonist. Intravenous infusion of adenosine (30 μg/min per rat, for 5 min) prior to the 50-min occlusion improved the recovery of GFR, and this protection of GFR was blocked by SPT. Thus, both IPC and exogenous adenosine attenuated ischemic-reperfusion injury of the kidney. However, because three adenosine receptor antagonists failed to abolish the protective effect of IPC, there is no evidence to indicate that activation of adenosine receptors contributes to the IPC effect in the kidney.

Keywords: Acute renal failure, Ischemia, Reperfusion, Preconditioning, Adenosine

Ischemic preconditioning, induced by a brief episode of ischemia and reperfusion, renders the heart more tolerant to subsequent sustained ischemia (1). This phenomenon has been confirmed in various organs, including the brain (2), skeletal muscle (3, 4), liver (5), intestine (6) and lung (7). However, the effect of ischemic preconditioning on the kidney has not been observed until recently (8, 9).

A recent study by Riera et al. (10) showed that preconditioning with 5 to 15 min of ischemia followed by 10 min of reperfusion protected renal function (serum creatinine levels) against ischemic-reperfusion injury induced by the sustained ischemia. Cochrane et al. (11) reported that a very short period of preconditioning, with one to three cycles of 2-min ischemia followed by 5 min of reperfusion, protected renal function (BUN and serum creatinine levels) and morphology. A slightly longer period of ischemic preconditioning, consisting of three cycles of 5-min ischemia, was not protective. In contrast, Lee and Emala (12) reported that preconditioning with four cycles of 8 min but not 6 min of ischemia protected renal function (BUN and plasma creatinine levels). Thus, it can be safely concluded that protection by ischemic preconditioning exists in the kidney. However, it is unknown whether multiple types of ischemic preconditioning, resulting from the different duration of the preconditioning periods, play a role in the kidney.

Numerous studies have been carried out to elucidate the mechanism of ischemic preconditioning; and special attention has been given to the finding that adenosine A1 receptors mediate the cardioprotective effect of preconditioning (13–15). At present, only one study using the long duration of multiple cycled preconditioning has suggested the role of adenosine in ischemic preconditioning in the kidney (12).

In all previous studies on a brief episode of renal ischemic preconditioning, the protection was assessed functionally and histologically at 24 h or more after sustained ischemia. There is no functional or morphologic information on the protective effect of such preconditioning at an early phase
of ischemic-reperfusion injury. Therefore, the present study assessed the early effects of a relatively short period of renal ischemic preconditioning in rats by functional, hemodynamic and histologic methods and examined whether activation of adenosine receptors contributes to renal protection induced by ischemic preconditioning.

MATERIALS AND METHODS

Animals and drugs

Male Wistar rats weighing 280–300 g were used. The animals were housed in a temperature-controlled (23 ± 1°C) and humidity-controlled (55 ± 5%) room with free access to food and water. 8-(p-Sulfophenyl)-theophylline (SPT), 1,3-dipropyl-8-cyclopentylxanthine (DPCPX), 3,7-dimethyl-1-propargylxanthine (DMPX) and adenosine were obtained from RBI® (Natick, MA, USA). Inulin was from Nacalai Tesque (Kyoto). SPT was dissolved in 0.08 N NaOH immediately before use (pH 6 to 7). DPCPX and DMPX were dissolved in 50% dimethylsulfoxide (DMSO) in saline. Adenosine was dissolved in saline.

Surgical preparation

Rats were anesthetized with a subcutaneous injection of urethane (1.24 g/kg) and placed on a warm plate (34°C). A tracheotomy was performed and an endotracheal tube was inserted to allow for spontaneous breathing. Both jugular veins were exposed for injection of drugs and blood sampling. The right femoral vein was catheterized for urine sampling. The urinary bladder was catheterized for urine sampling.

Systemic mean blood pressure was measured from the right femoral artery with a pressure transducer (P23XL; Ohmeda, Liberty Corner, NJ, USA), which was connected to a blood pressure amplifier (AP-641G; Nihon Kohden, Tokyo). Heart rate was measured with a heart rate counter (AT601G, Nihon Kohden). Renal blood flow was measured with an ultrasonic pulsed Doppler flowmeter (Model VF-1; Crystal Biotech, Holliston, MA, USA) by fitting a flow probe (inner diameter = 1 millimeter) around the left renal artery. Left renal artery occlusion was performed using a small vascular clip while taking care not to release the flow probe from the artery. Occlusion and reperfusion were confirmed by instantaneous changes in renal blood flow. The right kidney was left in place, in order to prevent retention of drugs during the ischemic period.

After surgical preparation, a period of 90 min was allowed for stabilization.

Experimental protocols

Three sets of experiments were performed and all rats were subjected to 50 min of renal artery occlusion followed by 120 min of reperfusion.

In the first set of experiments, the effect of the non-selective adenosine receptor antagonist SPT on ischemic preconditioning was examined by functional, hemodynamic, and histologic methods. The animals were assigned to four groups (n = 9 for each group). Group 1 (vehicle control group) received saline as the vehicle 20 min before 50 min of occlusion. Group 2 (IPC group) was preconditioned and received saline 4 min before preconditioning. Group 3 (SPT group) received SPT 20 min before 50 min of occlusion. Group 4 (SPT+IPC group) received SPT as in group 3, 4 min before preconditioning. Preconditioned rats were subjected to two cycles of 3 min of occlusion followed by 5 min of reperfusion. SPT (10 mg/kg) and saline were administered intravenously in a volume of 0.33 ml/kg.

In the second set of experiments, the effects of the selective A1-receptor antagonist DPCPX and the selective A2-receptor antagonist DMPX on ischemic preconditioning were examined. The animals were assigned to six groups (n = 5 for each group). Group 1 (vehicle control group) received 50% DMSO/saline as the vehicle 31 min before 50 min of occlusion. Group 2 (IPC group) was preconditioned and received 50% DMSO/saline 15 min before preconditioning. Group 3 (DPCPX group) received DPCPX 31 min before 50 min of occlusion. Group 4 (DPCPX+IPC group) received DPCPX as in group 3, 15 min before preconditioning. Group 5 (DMPX group) received DMPX 31 min before 50 min of occlusion. Group 6 (DMPX+IPC group) received DMPX as in group 5, 15 min before preconditioning. Preconditioned rats were subjected to two cycles of 3 min of occlusion followed by 5 min of reperfusion. DPCPX (1 mg/kg), DMPX (1 mg/kg) and 50% DMSO/saline were administered intraperitoneally in a volume of 0.33 ml/kg. Administration of 50% DMSO/saline produced a transient fall in systemic blood pressure and renal blood flow, which returned to basal levels within 3 min.

The present schedule for preconditioning was designed and tested, with reference to the schedules reported in previous studies, in which a relatively short duration of preconditioning was used: three cycles of 2 (11), 3 (16) or 5 (17, 18) min of preconditioning ischemia followed by 5 min of reperfusion. We measured blood flow in the left renal artery using the ultrasonic pulsed Doppler method. Therefore, to minimize the risk of release of the flow probe from the artery, resulting from frequent placement and release of the vascular clip for occlusion and reperfusion, two rather than three cycles of ischemia and reperfusion were selected.

In the third set of experiments, the effect of infused
adenosine on ischemic-reperfusion injury was evaluated in terms of renal function, using the following experimental groups: control (n = 5), adenosine (n = 5) and SPT+adenosine (n = 3) groups. Control animals received intravenous infusion of saline at a dose of 20 μl/min per rat for 5 min, before the 50 min of occlusion. In two adenosine-treated groups, the animals received adenosine at a dose of 30 μg/min per rat for 5 min, before the 50 min of occlusion. Infusion of adenosine induced a reduction of systemic blood pressure to the same extent, approximately 10 mmHg, as shown during the period of preconditioning reperfusion (data not shown). SPT (10 mg/kg) was administered intravenously before adenosine.

Measurements of hemodynamics

Systemic blood pressure, heart rate and renal blood flow were monitored throughout the experiments. During one 30-min basal period (i.e., a period before the treatment with the drug or vehicle) and during four consecutive 30-min reperfusion periods (i.e., periods from 0 to 30 min, from 30 to 60 min, from 60 to 90 min and from 90 to 120 min after the onset of reperfusion), hemodynamic parameters were recorded twice at 10-min intervals during each period. Then, the average value was calculated for each period. To estimate hemodynamic changes during ischemic preconditioning, the parameters were recorded 4 min after the onset of the first and second cycles of preconditioning reperfusion, and in the non-preconditioned groups, they were recorded at the corresponding time points.

Clearance study

Inulin clearance was performed for estimation of glomerular filtration rate (GFR). A 30-min urine sample was collected twice in one study: during the 30-min basal period and again during the period from 90 to 120 min after the onset of reperfusion. A blood sample was taken from the jugular vein at the mid-point of the urine-collection periods, and plasma was obtained by centrifugation. Inulin in urine and plasma was quantitated by the fluorometric method of Vurek and Pegram (19).

Histological evaluation

Histological evaluation was performed during the preconditioning study. After the completion of the clearance study, the left kidney was removed and cut transversely. The slices were fixed in buffered formalin and embedded in paraffin. Since red blood cells stained with formalin are a dark brown color, the area occupied by red blood cells in the ischemic kidney is demarcated from that with no blood trapping. To assess the severity of vascular congestion and/or hemorrhage, we measured the area of blood trapping. Briefly, the surface of the tissue embedded in paraffin was photographed, the dark area of the tissue was measured using software for image analysis (NIH Image), and the percentage of the dark area to the total area of a cross-section of the kidney was calculated. In our preliminary study, we found a positive and definite correlation (r = 0.91, n = 20) between the quantitative assessment by planimetry using image analysis and the semiquantitative assessment by light microscopy (data not shown). We did not distinguish between congestion and hemorrhage.

Subsequently, the tissues were sectioned and stained with hematoxylin and eosin. The sections were reviewed in a blind fashion for pathological assessment of renal injury. The number of casts and the number of proximal tubules with desquamation of tubular cells into the lumen, in 4 fields in the outer strip of the outer medulla (×200 magnification), were determined. A total of 4 fields were counted and the severity of morphologic damage per animal was graded from 0 (no changes) to 4 (severe changes).

Statistical analyses

The values were expressed as means ± S.E.M. Statistical analyses were performed by the paired Student’s t-test, unpaired Student’s t-test or Welch’s t-test and by one-way analysis of variance (ANOVA) followed by Fisher’s LSD test. Non-parametric analysis (Mann-Whitney U-test or Kruskal-Wallis test) was used for histological data. A value of P<0.05 was considered statistically significant.

RESULTS

Effects of SPT on ischemic preconditioning

Hemodynamic findings are summarized in Table 1. The IPC group did not show any changes in systemic blood pressure or heart rate from basal levels, throughout the preconditioning process; in contrast, the SPT+IPC group showed hypertension and tachycardia. However, there was no significant difference in renal blood flow between the IPC and SPT+IPC groups during the periods of the first and second cycles of preconditioning reperfusion. The pattern of changes after sustained ischemia in systemic blood pressure and that in heart rate did not differ significantly between the IPC and SPT+IPC groups. No reduction in renal blood flow after the sustained ischemia was clearly observed. Renal blood flow recovered to the pres ischemic levels by 60 min after the onset of reperfusion in all groups.

Mild polyuria developed after the sustained ischemia, and the degree of severity of polyuria did not differ significantly among the four groups (Table 1).

The control group showed a significant reduction in GFR during the period from 90 to 120 min after the onset of reperfusion (P<0.05 vs the basal values). Recovery of GFR is shown in Fig. 1. The data are expressed as a percentage of the basal values (basal values: 1.84 ± 0.14, 1.58 ± 0.11, 1.77 ± 0.12 and 1.65 ± 0.12 ml/min in the
Recovery of GFR was 87.1 ± 5.1%, 105.0 ± 4.5%, 83.2 ± 5.6% and 100.7 ± 4.6% in the control, IPC, SPT and SPT+IPC groups, respectively. Thus, ischemic-reperfusion injury led to slight recovery of GFR, and ischemic preconditioning significantly improved the recovery of GFR (P<0.05 vs the control group). SPT did not block the protective effect of ischemic preconditioning on GFR.

The histological findings are summarized in Fig. 2. At 120 min after reperfusion, no necrosis was apparent. However, there were conspicuous findings as follows: blood trapping, cast formation and desquamation of cells into the tubular lumen. When the size of the area of trapped red blood cells was expressed as a percentage of each kidney, the area of blood trapping in the control, IPC, SPT and SPT+IPC groups was 38.0 ± 4.3%, 22.6 ± 5.0%, 36.0 ± 4.1% and 22.0 ± 3.2%, respectively. Ischemic preconditioning significantly decreased the size of the area of blood trapping (P<0.05 vs the control group). SPT did not block the preventive effect of ischemic preconditioning on blood trapping. The pattern of histological scores for the tubular damage was similar to that of blood trapping. Ischemic preconditioning appeared to decrease histological scores for cast formation, and it significantly decreased those for desquamation of tubular cells (P<0.05 vs the control, IPC and SPT groups, respectively).

Table 1. Effects of SPT on blood pressure, heart rate, renal blood flow and urine flow

|                         | Basal value | Reperfusion during IPC | Reperfusion after 50-min ischemia |
|-------------------------|-------------|------------------------|----------------------------------|
|                         |             | 1st cycle | 2nd cycle | 0 – 30 min | 30 – 60 min | 60 – 90 min | 90 – 120 min |
| Blood pressure (mmHg)   |             |           |           |           |            |            |            |
| Control                 | 113 ± 3     | –2 ± 1    | 2 ± 2     | 16 ± 2    | 14 ± 2     | 13 ± 3     | 10 ± 2     |
| IPC                     | 114 ± 3     | 2 ± 3     | 6 ± 1     | 12 ± 2    | 12 ± 2     | 10 ± 2     | 7 ± 2      |
| SPT                     | 113 ± 4     | 10 ± 2    | 9 ± 2     | 14 ± 3    | 15 ± 4     | 14 ± 4     | 10 ± 3     |
| SPT+IPC                 | 114 ± 3     | 13 ± 2    | 13 ± 4    | 13 ± 2    | 12 ± 2     | 8 ± 2      | 4 ± 2      |
| Heart rate (bpm) (%)    |             |           |           |           |            |            |            |
| Control                 | 438 ± 10    | 0 ± 3     | 2 ± 3     | –3 ± 6    | –3 ± 4     | –7 ± 4     | –12 ± 6    |
| IPC                     | 440 ± 11    | 2 ± 3     | 5 ± 3     | 0 ± 3     | –4 ± 4     | –10 ± 4    | –13 ± 6    |
| SPT                     | 443 ± 12    | 4 ± 4     | 10 ± 4    | –10 ± 3   | –8 ± 3     | –15 ± 3    | –13 ± 4    |
| SPT+IPC                 | 428 ± 9     | 16 ± 3    | 20 ± 3    | –3 ± 5    | –6 ± 5     | –4 ± 5     | –6 ± 5     |
| Renal blood flow (kHz)  |             |           |           |           |            |            |            |
| Control                 | 6.54 ± 0.49 | 104.7 ± 1.6| 105.2 ± 1.4| 94.4 ± 4.0| 102.1 ± 4.4| 102.8 ± 3.7| 99.1 ± 4.3|
| IPC                     | 6.05 ± 0.38 | 98.3 ± 2.3 | 96.7 ± 2.5 | 92.1 ± 5.3| 102.5 ± 5.2| 101.3 ± 4.2| 100.4 ± 4.7|
| SPT                     | 6.38 ± 0.40 | 97.2 ± 1.4 | 101.0 ± 2.3| 99.8 ± 6.6| 105.4 ± 5.2| 106.9 ± 4.0| 103.3 ± 4.1|
| SPT+IPC                 | 6.54 ± 0.37 | 97.5 ± 2.1 | 97.3 ± 1.8 | 88.9 ± 3.6| 101.8 ± 2.9| 102.6 ± 2.6| 98.9 ± 3.9|
| Urine flow (µl/min) (%) |             |           |           |           |            |            |            |
| Control                 | 8.50 ± 1.00 |          |          | 248.7 ± 23.0|          |            |            |
| IPC                     | 8.21 ± 1.3  |          |          | 269.6 ± 48.1|          |            |            |
| SPT                     | 8.15 ± 0.62 |          |          | 196.5 ± 28.4|          |            |            |
| SPT+IPC                 | 8.02 ± 1.14 |          |          | 228.6 ± 22.2|          |            |            |

The control and SPT groups were not subjected to ischemic preconditioning. Therefore, in these groups, hemodynamic changes before 50-min ischemia were recorded at the corresponding time points to those in preconditioned groups. Other terms for the experimental schedules are defined in the text. Blood pressure and heart rate during preconditioning and postischemic reperfusion are expressed as change from basal values. Recovery of renal blood flow and urine flow is expressed as percentage of basal values. Data are given as means ± S.E.M. (n = 9 for each group). a,b,c 0.05 vs the control group. *P<0.05 vs the control group.
Effect of DPCPX and DMPX on ischemic preconditioning

Effects of DPCPX and DMPX on ischemic preconditioning

- Hemodynamic findings are summarized in Table 2.
- DPCPX had no effect on hemodynamic parameters before sustained ischemia in the preconditioned or non-preconditioned group; in contrast, DMPX induced hypertension without altering heart rate, and tended to reduce renal blood flow. The pattern of changes after sustained ischemia in systemic blood pressure and that in heart rate did not differ significantly among all six groups. However, recovery of renal blood flow in the IPC, DPCPX, DPCPX+IPC and DMPX+IPC groups was greater than that observed in the control group. DMPX did not modify the recovery of renal blood flow.

- Urine flow in the control and DMPX groups did not change after the sustained ischemia, whereas polynuria developed in the DPCPX group and in three preconditioned groups, the IPC, DPCPX+IPC and DMPX+IPC groups. The DPCPX and DPCPX+IPC groups showed severe polynuria (Table 2).

- The control group showed a significant reduction in GFR during the period from 90 to 120 min after the onset of reperfusion (P<0.05 vs the basal values). Recovery of GFR was shown in Fig. 3. The data are expressed as a percentage of the basal values (basal values: 3.5% and 67.8% in the control, adenosine only and SPT+adenosine groups, respectively). Recovery of GFR was significantly improved by ischemic preconditioning (P<0.05 vs the control group). Neither DPCPX nor DMPX blocked the protective effect of ischemic preconditioning on GFR.

- The histological findings are summarized in Fig. 4. When the size of the area of trapped red blood cells was expressed as a percentage of each kidney, the area of blood trapping in the control, IPC, DPCPX, DPCPX+IPC, DMPX and DMPX+IPC groups was 32.7 ± 2.4%, 13.4 ± 2.4%, 32.5 ± 2.0%, 14.2 ± 2.4%, 29.3 ± 2.1% and 10.8 ± 2.8%, respectively. Ischemic preconditioning significantly decreased the size of the area of blood trapping (P<0.05 vs the control group). In addition, ischemic preconditioning significantly decreased histological scores for desquamation of tubular cells (P<0.05 vs the control group). Neither DPCPX nor DMPX blocked the effect of ischemic preconditioning on blood trapping or tubular damage.

Effects of infused adenosine

- The control group showed a significant reduction in GFR during the period from 90 to 120 min after the onset of reperfusion (P<0.05 vs the basal values). Recovery of GFR is shown in Fig. 5. The data are expressed as a percentage of the basal values (basal values: 2.14 ± 0.23, 1.82 ± 0.23 and 2.07 ± 0.10 ml/min in the control, adenosine and SPT+adenosine groups, respectively). Recovery of GFR was 74.3 ± 12.6%, 119.7 ± 6.9% and 67.8 ± 5.8% in the control, adenosine only and SPT+adenosine groups, respectively. Thus, exogenous adenosine prevented the ischemia-induced reduction of GFR (P<0.05 vs the control group). Moreover, the protective effect of adenosine on GFR was blocked by SPT pretreatment.

DISCUSSION

The present study examined the early effects of a relatively short period of renal ischemic preconditioning in rats, by functional, hemodynamic and histologic methods. Furthermore, we attempted to determine whether the adenosine receptor antagonists, SPT, DPCPX and DMPX, are capable of blocking such protection.

Renal ischemic-reperfusion injury is known to lead to a reduction in both GFR (20) and renal blood flow (21). Our results showed that GFR was slightly but significantly reduced after the sustained ischemia in the control groups in two experiments of ischemic preconditioning. The slight reduction in GFR was due to the fact that GFR values reflected the sum of renal function of the ischemic and control group). SPT did not block such protection induced by ischemic preconditioning of renal tubules.
contralateral healthy kidneys. We also showed that ischemic preconditioning improved the postischemic recovery of GFR. On the other hand, there were inconsistent results concerning recovery of renal blood flow: renal blood flow was not reduced in the control group that received saline as the vehicle, but that in the control group that received 50% DMSO/saline was markedly reduced. Although the reason for the different results for renal blood flow remains unclear, it may be due to different experimental conditions; for example, the vehicle (saline or DMSO/saline) and the schedule for administration.

Observed necrotic changes were somewhat mild, likely because morphology was investigated at an early phase of ischemic-reperfusion injury. On the other hand, marked blood trapping was favorable. The results of assessment of blood trapping closely reflected the protective effect of preconditioning. Necrosis of the proximal tubule was most evident at 2 days after ischemia of 30 min or longer (22), at which point tubular injury became less severe (8, 23). In addition, the recovery times of injured tubules were related to the severity of injury (22). Therefore, it may be predicted that a brief episode of ischemic preconditioning can produce earlier recovery of tubules, likely due to the attenuated severity of injury and resulting enhanced regeneration of cells.

Previous studies (8, 9) have suggested that ischemic preconditioning with a brief episode of ischemia and reperfusion is not effective in the kidney. However, recent studies (10 – 12), including the present one, have confirmed that there is ischemic preconditioning effect in the rat

### Table 2. Effects of DPCPX and DMPX on blood pressure, heart rate, renal blood flow and urine flow

| Blood pressure (mmHg) | Reperfusion during IPC | Reperfusion after 50-min ischemia |
|-----------------------|------------------------|----------------------------------|
| **Control**           | 107 ± 2                | 1 ± 2                            | 0 ± 2                            | 8 ± 2 | 7 ± 1 | 7 ± 2 | 3 ± 1 |
| **IPC**               | 107 ± 8                | 8 ± 1                            | 7 ± 1                            | 13 ± 8 | 6 ± 3 | 7 ± 4 | 2 ± 5 |
| **DPCPX**             | 107 ± 4                | 7 ± 3                            | 1 ± 3                            | 12 ± 1 | 10 ± 2 | 7 ± 1 | 5 ± 2 |
| **DPCPX+IPC**         | 111 ± 7                | 7 ± 4                            | 6 ± 7                            | 11 ± 4 | 6 ± 3 | 7 ± 1 | 4 ± 2 |
| **DMPX**              | 111 ± 3                | 15 ± 2 (a)                       | 11 ± 1                           | 11 ± 2 | 8 ± 3 | 8 ± 3 | 2 ± 2 |
| **DMPX+IPC**          | 110 ± 5                | 13 ± 3 (a)                       | 12 ± 3                           | 10 ± 3 | 10 ± 1 | 7 ± 3 | 9 ± 3 |

| Heart rate (bpm)      | Reperfusion during IPC | Reperfusion after 50-min ischemia |
|-----------------------|------------------------|----------------------------------|
| **Control**           | 440 ± 4                | 4 ± 2                            | 8 ± 3                            | −11 ± 3 | −9 ± 2 | −9 ± 4 | −16 ± 2 |
| **IPC**               | 447 ± 3                | 1 ± 1                            | 0 ± 1                            | −2 ± 6 | −6 ± 5 | −9 ± 2 | −11 ± 2 |
| **DPCPX**             | 444 ± 4                | 1 ± 3                            | 0 ± 1                            | −3 ± 2 | −7 ± 4 | −7 ± 3 | −9 ± 3 |
| **DPCPX+IPC**         | 443 ± 3                | 7 ± 2                            | 7 ± 2                            | −3 ± 2 | −5 ± 2 | −9 ± 3 | −12 ± 4 |
| **DMPX**              | 446 ± 6                | −1 ± 6                           | 4 ± 7                            | −9 ± 2 | −9 ± 3 | −15 ± 1 | −18 ± 4 |
| **DMPX+IPC**          | 448 ± 6                | 0 ± 3                            | 1 ± 4                            | −5 ± 4 | −8 ± 3 | −12 ± 4 | −12 ± 5 |

| Renal blood flow (kHz) | Reperfusion during IPC | Reperfusion after 50-min ischemia |
|------------------------|------------------------|----------------------------------|
| **Control**           | 5.85 ± 0.46            | 102.2 ± 2.3                       | 103.5 ± 2.6                       | 65.2 ± 6.4 | 72.2 ± 5.6 | 74.9 ± 5.9 | 70.6 ± 5.7 |
| **IPC**               | 5.57 ± 0.20            | 111.5 ± 3.6                       | 110.0 ± 4.0                       | 91.2 ± 5.3 (a) | 99.7 ± 5.5 (a) | 102.4 ± 6.6 (a) | 95.6 ± 6.5 (a) |
| **DPCPX**             | 5.91 ± 0.36            | 102.2 ± 6.0                       | 96.8 ± 4.9                       | 91.1 ± 2.5 (a) | 96.6 ± 3.4 (a) | 96.7 ± 3.6 (a) | 92.2 ± 4.7 (a) |
| **DPCPX+IPC**         | 5.75 ± 0.18            | 105.0 ± 5.4                       | 99.2 ± 4.5                       | 86.7 ± 3.4 (a) | 97.8 ± 2.6 (a) | 99.1 ± 3.2 (a) | 93.1 ± 1.9 (a) |
| **DMPX**              | 5.69 ± 0.39            | 93.7 ± 3.0                        | 92.3 ± 3.9                       | 78.8 ± 6.2 | 80.3 ± 6.7 | 81.1 ± 7.2 | 72.9 ± 6.3 |
| **DMPX+IPC**          | 5.89 ± 0.40            | 99.3 ± 3.6                       | 97.4 ± 3.6 (a) | 92.3 ± 5.4 (a) | 99.2 ± 4.9 (a) (c) | 99.7 ± 5.2 (a) (c) | 92.7 ± 3.9 (a) (c) |

| Urine flow (µl/min)   | Reperfusion during IPC | Reperfusion after 50-min ischemia |
|-----------------------|------------------------|----------------------------------|
| **Control**           | 8.22 ± 0.95            |                                   |                                  | 130.0 ± 62.6 |
| **IPC**               | 7.10 ± 1.14            |                                   |                                  | 215.4 ± 61.8 |
| **DPCPX**             | 7.84 ± 1.56            |                                   |                                  | 315.6 ± 70.2 (a) |
| **DPCPX+IPC**         | 7.82 ± 1.28            |                                   |                                  | 373.3 ± 39.2 (a) (b) |
| **DMPX**              | 8.31 ± 1.80            |                                   |                                  | 159.1 ± 43.9 |
| **DMPX+IPC**          | 7.42 ± 1.71            |                                   |                                  | 266.2 ± 24.4 |

The control, DPCPX and DMPX groups were not subjected to ischemic preconditioning. Therefore, in these groups, hemodynamic changes before 50-min ischemia were recorded at the corresponding time points to those in preconditioned groups. Other terms for the experimental schedules are defined in the text. Blood pressure and heart rate during preconditioning and postischemic reperfusion are expressed as change from basal values. Recovery of renal blood flow and urine flow is expressed as percentage of basal values. Data are given as means ± S.E.M. (n = 5 for each group). (a), (b) and (c): P<0.05 vs the control, IPC and DMPX groups, respectively.
kidney. The discrepancies between the results of the earlier and more recent studies may have arisen from the different preconditioning schedules. Indeed, renal protection has been observed in rats subjected to 5-min or 10-min intervals between the ischemic preconditioning and the sustained ischemia (10, 11), but not among rats subjected to longer intervals, such as 30 min (8, 9). In the study by Lee and Emala (12), the preconditioning effect in the kidney lasted for up to 60 min. This finding may be related to the fact that they evaluated the effect of preconditioning using four cycles of 8-min ischemia, which was the longest duration of cycled preconditioning ischemia among the reported preconditioning schedules. However, no evidence is presently available to indicate that longer durations of cycled preconditioning ischemia leads to a more sustained effect of preconditioning; namely, the longer critical interval after preconditioning.

Studies on the mechanisms of myocardial ischemic preconditioning have suggested that the protective effect of preconditioning in dog (14) and pig (15) hearts is mediated by adenosine A₁ receptors, whereas that in the rabbit heart is mediated by both adenosine A₁ receptors (13, 24) and, in part, by A₃ receptors (25). Li and Kloner (16) reported that the preconditioning effect in the rat heart was not mediated by adenosine receptors in vivo. However, adenosine improves postischemic myocardial function via an adenosine A₁ receptor mechanism in vitro (26). Also, preconditioning in the rat liver is reportedly mediated by adenosine A₂ receptors (27). Thus, recent evidence suggests that activation of adenosine receptors plays a role in protection induced by ischemic preconditioning. However, the mechanisms of renal protection remain unclear.

To confirm whether preconditioning in the kidney is related to adenosine receptor activation induced by adenosine release during preconditioning, we first examined the effect of SPT, a non-selective adenosine receptor antagonist. Because SPT has a short half-life of 10 min (16), and is quickly eliminated from the tissues by perfusion (28), its use is advantageous in one respect in that it avoids the situation whereby the antagonist is left in the blood during
the ischemic-reperfusion period. Of course, the possibility exists that SPT is unable to block adenosine receptor activation during preconditioning because of a short duration of action.

In the SPT+IPC group, SPT pretreatment induced hypertension and tachycardia during the second cycle of preconditioning reperfusion. SPT has been reported to increase vascular resistance (29). In addition, A1 agonist produced hypotension and bradycardia, and A2 agonist produced hypotension and tachycardia (12). Hypertension observed in our study is due to the blockade of adenosine receptors. Although we have no direct evidence that SPT blocked only adenosine A1 receptors, tachycardia is evidently due to the blockade of A1-receptor activation induced by adenosine release during preconditioning. Hence, tachycardia is likely mediated by A2 receptors. Taken together, these findings suggested that the action of SPT lasted during preconditioning. We found that such pretreatment with SPT did not block the protective effect of ischemic preconditioning on renal function (GFR) or morphology.

We next examined the effects of the selective A1-receptor antagonist DPCPX and the selective A2-receptor antagonist DMPX. Administration of DPCPX to non-preconditioned rats led to similar recovery of renal blood flow as that observed in the IPC group, and led to more severe polyuria than that observed in the control or IPC group. Because DPCPX is diuretic (30) and the blockade of A1 receptors by DPCPX persists for at least 5 h (31), our findings seem to indicate that the action of DPCPX lasts during reperfusion. However, as in the case of SPT, DPCPX failed to block the protective effects on GFR and renal morphology. In addition, we found that DMPX, which was reported to block the protective effect of hepatic ischemic preconditioning (27), completely failed to modify the preconditioning effect in the rat kidney.

Adenosine infusion before the sustained ischemia improved the recovery of GFR. The effect of adenosine on GFR, unlike that of ischemic preconditioning, was blocked by SPT pretreatment. Our findings during the 120-min of reperfusion supported those of another study observed at 24 h after sustained ischemia (12), which showed that exogenous adenosine protected renal function (BUN and plasma creatinine levels) via the adenosine A1-receptor mechanism and that DPCPX failed to block the protective effect on renal function of ischemic preconditioning. Activation of A2 receptors as well as A1 receptors was not involved in renal protection induced by exogenous adenosine (12).

Indeed, we did not show that activation of adenosine receptors contributed to renal protection induced by ischemic preconditioning in the kidney. However, because the protective effect of infused adenosine was completely blocked by SPT, it is likely that multiple mediators (including at least adenosine) play a role in renal preconditioning, concomitantly and independently, similar to the case of hypoxic preconditioning in rat cardiomyocytes (32).

Lee and Emala (12) reported that four cycles of 6-min ischemia was an insufficient duration to induce renal protection. In contrast, Cochrane et al. (11) contended that preconditioning with one to three cycles of 2-min ischemia was renal protective, whereas three cycles of 5-min ischemia was not. The results of our study, in terms of the role of adenosine in renal protection induced by ischemic preconditioning, using a shorter period of preconditioning ischemia were fundamentally in accord with those of the study using a longer period of preconditioning ischemia. Accordingly, the existence of a common mechanism underlying the different durations of renal ischemic preconditioning may be suggested; although further studies are needed to elucidate the mechanism involved in ischemic preconditioning in the kidney.

In summary, the present study confirmed that a brief episode of ischemia and reperfusion attenuated ischemic-reperfusion injury of the rat kidney, at an early phase of ischemic-reperfusion injury. Exogenous adenosine mimicked ischemic preconditioning. The renal protection induced by infused adenosine was blocked by the non-selective adenosine receptor antagonist SPT. However, SPT, the selective A1-receptor antagonist DPCPX and the selective A2-receptor antagonist DMPX failed to abolish the protective effect of ischemic preconditioning. Therefore, there was no evidence to indicate that activation of adenosine receptors contributed to renal protection induced by ischemic preconditioning in the kidney.

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