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Connexins 37 and 40 transduce purinergic signals mediating renal autoregulation

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The kidney exhibits a remarkable capacity to maintain renal blood flow and glomerular filtration rate constant in the face of marked variations in systemic blood pressure (28, 37). There is a consensus that both the myogenic response and tubuloglomerular feedback (TGF) are required for efficient renal autoregulation. Although myogenic responses are intrinsic to vascular smooth muscle cells and present in various vascular beds, including cerebral, mesenteric, and coronary arteries (6, 44, 45), the TGF is specific to the kidney (27, 28, 34). Loutzenhiser et al. (21) denoted that afferent arteriolar myogenic constriction is determined by systolic but not mean blood pressure. However, recent analyses of the dynamics of renal autoregulation reveal that the two mechanisms interact with each other (16, 50). Because TGF constrains the terminal portion of the afferent arteriole (28, 37), it increases upstream pressure, enhancing myogenic constriction and influencing the autoregulatory capacity of adjacent nephrons (13, 17). Furthermore, the TGF mediator induces oscillations in tubular pressure (3, 19, 41).

Recent progress in research on the mediator of TGF is impressive. A cornerstone study by Bell et al. (1) demonstrated that macula densa cells release ATP through maxi-anion channels. However, the debate on how TGF signals transduce to the afferent arteriole fails to provide a consistent pattern. On the one hand, Inscho et al. (14) demonstrated that TGF is markedly impaired in juxtamedullary nephrons of purinergic receptor knockout mice. Sun et al. (36) reported the absence of TGF in A1 receptor knockout mice. In addition, the data of Thomson et al. (47) and ours (42) suggest that 5'-nucleotidase plays a role in mediating TGF. More recently, Peti-Peterdi (29) conducted an elegant study demonstrating that activation of TGF increased cytosolic calcium in the extraglomerular mesangium and the juxtaglomerular renin granular cells. This calcium wave propagated to the proximal segment of the afferent arteriole and to the glomerular podocytes and could be blocked by the putative gap junction uncouplers, heptanol or alphaglycyrrhetic acid (29). Indeed, heptanol inhibited the TGF-induced afferent arteriolar constriction (30). Six connexins (Cx) oligomerize to form a connexon or hemichannel in the plasma membrane (31). At least 20 Cxs have been cloned. Docking with a counterpart from an adjacent cell creates a gap junction. In addition to forming gap junctions, Cx hemichannels can play physiological roles in the release of ATP and NAD (32). To our knowledge, however, no data are available concerning which Cx subtypes are involved in TGF. Our previous study indicated that Cx37 and Cx40 were expressed in the juxtaglomerular apparatus, but Cx43 was localized within the glomerulus (39). In the present study, we assessed the effects on renal autoregulation of GAP peptides, which are reported...
to block specific Cxs through mimicry of extracellular sequences.

METHODS

Experiments were performed using 8- to 10-wk-old male Wistar-Kyoto rats (Shizuoka Laboratory Animal Center, Shizuoka, Japan) with approval from the ethical committee of the Saitama Medical College. Animals had free access to tap water and standard rat chow (CE-2, Nihon CLEA, Tokyo, Japan).

On the day of the experiment, the rats were anesthetized with pentobarbital sodium (50 mg/kg ip) and placed on a thermostatically controlled heated table to keep body temperature at 37°C, as detailed previously (40, 42). A tracheostomy was performed, and the right jugular vein was cannulated with polyethylene (PE-50) tubing to allow infusion of solutions and additional anesthetic. The animals were infused at the rate of 1.2 ml/h with isotonic saline solution containing 6% BSA (Sigma-Aldrich, St. Louis, MO) during surgery and thereafter with isotonic saline solution containing 1% BSA, 7.5% Inulet (Laevosan-Gesellschaft, Linz/ Donau, Austria) and 1.5% PAH (Merck Sharp & Dohme, West Point, PA). The left femoral artery was catheterized with PE-50 filled with heparinized saline (100 U/ml) to allow blood sampling and continuous arterial pressure measurements with a transducer (DX-100, Nihon Kohden, Tokyo, Japan) and a polygraph recorder (RM-7000, Nihon Kohden).

Mean arterial pressure (MAP) was used as renal arterial pressure. There was no irregularity of the arterial pulse throughout experimental periods. The abdomen was opened by a midline incision. The left ureter was cannulated (PE-10), and urine was collected under mineral oil in preweighed tubes. An adjustable clamp was placed on the aorta above the left renal artery to control left renal arterial pressure. For GAP peptide experiments, the left adrenal artery was cannulated with PE-10 to infuse heparinized saline or GAP peptides (5 mg ia followed by 0.2 mg/min) at a rate of 0.6 ml/h (5, 42, 43), and the solution for transjugal infusion was adjusted to that containing 2% BSA and infused at the rate of 0.6 ml/h to make water load similar. In the case of Cx40GAP27, saline containing acetic acid was used as a vehicle in the control and experimental periods. As many of the putative gap junction antagonists have been reported to have nonspecific effects (23, 46), we used the GAP peptides to probe physiological function. These peptides are reported to block specific Cxs through mimicry of extracellular sequences (8, 22). Rats were allowed to breathe air enriched with oxygen (100% O2), which markedly improves the stability of arterial blood pressure. After completion of surgery, 1 h of equilibration was allowed before initiating experimental protocols.

In the first study, to test whether Cxs may participate in renal autoregulation, the effects of three different GAP peptides, Cx40GAP26, Cx40GAP27, and Cx37,43GAP27, alone or in the presence of pyridoxal-phosphate-6-azophenyl-2,4-disulfonic acid (PPADS), a selective adenosine-1 receptor antagonist (Sigma), were examined. This series of studies comprised three groups of rats (6 rats per group) and was conducted in the same manner as for the previous studies with PPADS, testing responses to a pressure drop of 20 mmHg. CPX was intravenously administered (0.3 mg/kg) and repeated every 20 min (35, 42). Effects of GAP peptides on blood pressure were prevented by adjustment of the aortic clamp as before. The stock solution of CPX (2.5 mg/ml) was freshly prepared with 0.1 M NaOH in saline on the day of each experiment (42).

In complementary studies, the effects on renal autoregulation of CPX and PPADS alone and in combination were examined. This series of studies also contained three groups, with 6 rats in each group. Study protocol was again similar to that described above. The first autoregulation test was performed in the presence of CPX, PPADS, or both, while in the subsequent test, the isotonic saline solution contained CPX, PPADS, or both, along with 30% BSA, 7.5% Inulet, 1.5% PAH, and furosemide (16 μg·kg⁻¹·min⁻¹) to inhibit TGF without changes in blood pressure (26, 42).

In the third group, the influence of combined treatment with Cx40GAP27 and Cx37,43GAP27 on renal autoregulation was assessed. Two consecutive 20-min control clearances were carried out. The aortic clamp was tightened to reduce renal arterial pressure approximately by 20 mmHg before initiating two consecutive 20-min clearance periods. Subsequently, the aortic clamp was released. Saline was exchanged to that containing GAP peptides and infused into the adrenal artery throughout the remaining experimental periods. Aortic clamp was slightly tightened to maintain renal arterial pressure at the control level. Another 20-min equilibration period was allowed before initiating two consecutive 20-min clearance periods. Subsequently, the aortic clamp was further tightened to reduce renal arterial pressure approximately by 20 mmHg, and two consecutive 20-min clearance studies were carried out. Finally, aortic clamp was fully released, and losartan (10 mg/kg) was intravenously administered (40). Autoregulatory behavior was again examined in the presence of Cx40GAP27, Cx37,43GAP27, and losartan.

Arterial blood samples (~0.2 ml) were taken at the midpoint of each clearance period. Cells were separated by centrifugation, and plasma was removed. Urine volume was determined gravimetrically. Inulin and PAH concentrations in both plasma and urine were measured by standard spectrophotometry to enable calculation of effective renal plasma flow (RPF) and glomerular filtration rate (GFR). At the end of experiment, the rats were killed with an overdose of pentobarbital, and the left kidney was removed, decapsulated, blotted dry, and weighed. Because the GAP peptides may alter basal RPF and GFR, autoregulatory capacity was compared using autoregulatory index (AI), calculated according to following formulas:

For RPF:

\[
A1 \text{ for RPF} = \frac{\text{RPF}_{1}}{\text{RPF}_{2}} \times \frac{\text{MAP}_{2} - \text{MAP}_{1}}{\text{MAP}_{1}}
\]

For GFR:

\[
A1 \text{ for GFR} = \frac{\text{GFR}_{2} - \text{GFR}_{1}}{\text{GFR}_{1}} \times \frac{\text{MAP}_{2} - \text{MAP}_{1}}{\text{MAP}_{1}}
\]

RPF1, GFR1, and MAP1 indicate those at control MAP. RPF2, GFR2, and MBP2 depict those at reduced MAP.

Data were expressed as means ± SE. Statistical analysis was performed, using ANOVA and Student’s t-test with or without Bonferroni correction. P < 0.05 was considered to be statistically significant.

RESULTS

Effects of Cx37,43GAP27, PPADS, and CPX on autoregulatory indices of RPF and GFR. Under control conditions, RPF (4.07 ± 0.24 to 4.00 ± 0.31 ml·min⁻¹·g kidney⁻¹, n = 6) and GFR (1.02 ± 0.03 to 1.00 ± 0.03 ml·min⁻¹·g kidney⁻¹, n = 6) were measured by standard spectrophotometry to enable calculation of effective renal plasma flow (RPF) and glomerular filtration rate (GFR). At the end of experiment, the rats were killed with an overdose of pentobarbital, and the left kidney was removed, decapsulated, blotted dry, and weighed. Because the GAP peptides may alter basal RPF and GFR, autoregulatory capacity was compared using autoregulatory index (AI), calculated according to following formulas: A1 for RPF = ([RPF2 − RPF1]/[RPF1])/(MAP2 − MAP1)/MAP1) and A1 for GFR = ([GFR2 − GFR1]/GFR1)/(MAP2 − MAP1)/MAP1).

RPF1, GFR1, and MAP1 indicate those at control MAP. RPF2, GFR2, and MBP2 depict those at reduced MAP.

Data were expressed as means ± SE. Statistical analysis was performed, using ANOVA and Student’s t-test with or without Bonferroni correction. P < 0.05 was considered to be statistically significant.
were well autoregulated in the range of MAP from 103 ± 2 to 82 ± 2 mmHg (Fig. 1, A and B). Following intrarenal infusion of Cx37,43GAP27, autoregulation of RPF (3.27 ± 0.21 to 2.93 ± 0.19 ml·min⁻¹·g kidney wt⁻¹, P < 0.05) and GFR (1.02 ± 0.02 to 0.91 ± 0.02 ml·min⁻¹·g kidney wt⁻¹, P < 0.05) was impaired between 103 ± 2 and 82 ± 1 mmHg. Thus, the autoregulatory index to RPF (0.04 ± 0.06 to 0.50 ± 0.05, P < 0.05) and GFR (0.03 ± 0.07 to 0.50 ± 0.06, P < 0.05) was increased by Cx37,43GAP27 (Fig. 1, A and B). However, subsequent administration of PPADS did not further diminish the autoregulation of RPF (3.34 ± 0.23 to 2.98 ± 0.19 ml·min⁻¹·g kidney wt⁻¹) or GFR (0.95 ± 0.02 to 0.86 ± 0.02 ml·min⁻¹·g kidney wt⁻¹).

In the second group of rats, Cx37,43GAP27 and CPX were applied (Fig. 1, C and D). Under basal conditions, the reduction of MAP form 103 ± 3 to 81 ± 2 mmHg again failed to alter both RPF (4.31 ± 0.27 to 4.23 ± 0.32 ml·min⁻¹·g kidney wt⁻¹, n = 6) and GFR (1.04 ± 0.04 to 1.03 ± 0.04 ml·min⁻¹·g kidney wt⁻¹). During infusion of Cx37,43GAP27, RPF (3.48 ± 0.24 to 3.15 ± 0.24 ml·min⁻¹·g kidney wt⁻¹, P < 0.05) and GFR (1.05 ± 0.04 to 0.95 ± 0.04 ml·min⁻¹·g kidney wt⁻¹, P < 0.05) declined following a decrease in MAP.
(102 ± 2 to 81 ± 2 mmHg). In contrast to the effect of PPADS, the additional administration of CPX produced a further decline in autoregulation of RPF (3.35 ± 0.21 to 2.78 ± 0.18 ml·min⁻¹·g kidney wt⁻¹, P < 0.01) and GFR (1.02 ± 0.03 to 0.84 ± 0.03 ml·min⁻¹·g kidney wt⁻¹, P < 0.01) in response to a similar decrease in MAP of 101 ± 2 to 81 ± 2 mmHg, compared with Cx37,43GAP27 alone (Fig. 1, C and D).

Effects of Cx40GAP27, PPADS, and CPX on autoregulatory indices of RPF and GFR. RPF (4.33 ± 0.31 to 4.32 ± 0.33 ml·min⁻¹·g kidney wt⁻¹, n = 6) and GFR (1.02 ± 0.03 to 1.00 ± 0.03 ml·min⁻¹·g kidney wt⁻¹) were autoregulated in the range of MAP from 99 ± 2 to 81 ± 1 mmHg under control conditions. Following intrarenal infusion of Cx40GAP27, autoregulation of RPF (3.60 ± 0.26 to 3.20 ± 0.24 ml·min⁻¹·g kidney wt⁻¹, P < 0.05) and GFR (0.97 ± 0.03 to 0.87 ± 0.02 ml·min⁻¹·g kidney wt⁻¹, P < 0.05) was impaired between 102 ± 2 and 81 ± 1 mmHg. Thus, Cx40GAP27 increased autoregulatory indices of RPF and GFR (Fig. 2, A and B). Subsequent administration of PPADS, in addition to Cx40GAP27, did not produce any further change in autoregulation of RPF (3.65 ± 0.33 to 3.27 ± 0.31 ml·min⁻¹·g kidney wt⁻¹) or GFR (0.98 ± 0.03 to 0.87 ± 0.03 ml·min⁻¹·g kidney wt⁻¹) for a decrease in MAP of 101 ± 2 to 81 ± 2 mmHg (Fig. 2, A and B).

Fig. 2. Effects of GAP27 for connexin 40 (Cx40GAP27) on autoregulation of renal plasma flow (A, C) and glomerular filtration rate (B, D). * and #Significantly different from the value obtained in control and Cx40GAP27 period.

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Similar experiments were conducted for Cx40GAP27 and CPX (Fig. 2, C and D). Under basal conditions, the reductions of MAP from 101 ± 2 to 79 ± 1 mmHg failed to alter both RPF (4.63 ± 0.31 to 4.57 ± 0.35 ml·min⁻¹·g kidney wt⁻¹) and GFR (1.04 ± 0.03 to 1.03 ± 0.04 ml·min⁻¹·g kidney wt⁻¹). Thus, acetic acid did not affect renal autoregulation in the present study.

During infusion of Cx40GAP27, RPF (3.88 ± 0.26 to 3.55 ± 0.27 ml·min⁻¹·g kidney wt⁻¹, P < 0.05) and GFR (0.99 ± 0.02 to 0.90 ± 0.02 ml·min⁻¹·g kidney wt⁻¹, P < 0.05) decreased in response to change in MAP (99 ± 2 to 80 ± 1 mmHg). In the presence of Cx40GAP27 and CPX, a similar decrease in MAP from 102 ± 2 to 81 ± 2 mmHg produced a further decline in autoregulation of RPF (4.22 ± 0.27 to 3.51 ± 0.23 ml·min⁻¹·g kidney wt⁻¹, P < 0.01) and GFR (1.06 ± 0.03 to 0.89 ± 0.03 ml·min⁻¹·g kidney wt⁻¹, P < 0.01) with the autoregulatory indices to RPF and GFR significantly elevated during combination treatment with CPX and Cx40GAP27, compared with Cx40GAP27 alone (Fig. 2, C and D).

Effects of Cx43GAP26, PPADS, and CPX on autoregulatory indices of RPF and GFR. Figure 3 shows the effects of Cx43GAP26, PPADS, and CPX on autoregulatory indexes of RPF and GFR. As in the previous studies, RPF (4.68 ± 0.23 to 4.61 ± 0.27 ml·min⁻¹·g kidney wt⁻¹, n = 6) and GFR (1.02 ± 0.02 to 1.00 ± 0.03 ml·min⁻¹·g kidney wt⁻¹) were well autoregulated in the range of MAP from 100 ± 2 to 80 ± 1 mmHg. However, following intrarenal infusion of Cx43GAP26, autoregulation of RPF (4.48 ± 0.26 to 4.53 ±

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**Fig. 3.** Effects of GAP26 for connexin 43 (Cx43GAP26) on autoregulation of renal plasma flow (A, C) and glomerular filtration rate (B, D). *Significantly different from the control.
0.24 ml·min⁻¹·g kidney wt⁻¹) and GFR (0.93 ± 0.02 to 0.93 ± 0.02 ml·min⁻¹·g kidney wt⁻¹) was preserved between 98 ± 1 and 79 ± 2 mmHg. Subsequent administration of PPADS diminished autoregulation of RPF (4.52 ± 0.25 to 4.02 ± 0.19 ml·min⁻¹·g kidney wt⁻¹, P < 0.05) or GFR (0.95 ± 0.02 to 0.85 ± 0.02 ml·min⁻¹·g kidney wt⁻¹, P < 0.05) for change in MAP of 97 ± 1 to 78 ± 1 mmHg. Thus, autoregulatory indices of RPF and GFR were not altered by Cx43GAP26, but were increased by PPADS (Fig. 3, A and B).

Similar results were obtained for Cx43GAP26 and CPX. Under basal conditions, reduction of MAP form 99 ± 2 to 79 ± 2 mmHg failed to alter both RPF (4.37 ± 0.34 to 4.33 ± 0.40 ml·min⁻¹·g kidney wt⁻¹) and GFR (1.03 ± 0.04 to 1.02 ± 0.05 ml·min⁻¹·g kidney wt⁻¹). During infusion of Cx43GAP26, RPF (4.17 ± 0.33 to 4.16 ± 0.37 ml·min⁻¹·g kidney wt⁻¹) and GFR (0.94 ± 0.04 to 0.93 ± 0.04 ml·min⁻¹·g kidney wt⁻¹) remained unchanged despite a decrease in MAP (98 ± 2 to 80 ± 1 mmHg). The additional administration of CPX induced a decline in RPF (4.28 ± 0.30 to 3.83 ± 0.25 ml·min⁻¹·g kidney wt⁻¹, P < 0.05) and GFR (0.96 ± 0.04 to 0.87 ± 0.03 ml·min⁻¹·g kidney wt⁻¹, P < 0.05) in response to a decrease in MAP of 100 ± 1 to 81 ± 2 mmHg. Thus, autoregulatory indices of RPF and GFR were increased by CPX (Fig. 3, C and D).

Effects of GAP peptide on RPF and GFR at basal pressure. As summarized in Table 1, Cx46GAP27 and Cx37,43GAP27 induced a decrease in RPF without changes in GFR, suggesting preferential postglomerular vasoconstriction. In contrast, Cx43GAP26 reduced GFR with marginal decline in RPF. The latter is consistent with preferential preglomerular constriction or a decrease in ultrafiltration coefficient.

Effects of PPADS and CPX on autoregulatory indices of RPF and GFR. In complementary studies, the effects of PPADS and/or CPX on autoregulatory behavior of RPF and GFR were examined. Control studies again demonstrated good autoregulation of RPF (3.53 ± 0.49 to 3.52 ± 0.51 ml·min⁻¹·g kidney wt⁻¹, n = 6) and GFR (0.97 ± 0.11 to 0.97 ± 0.09 ml·min⁻¹·g kidney wt⁻¹) for a change in MAP from 101 ± 3 to 82 ± 3 mmHg. Subsequent administration of PPADS impaired autoregulation of RPF (3.78 ± 0.34 to 3.38 ± 0.28 ml·min⁻¹·g kidney wt⁻¹, P < 0.05) and GFR (1.14 ± 0.11 to 1.02 ± 0.09 ml·min⁻¹·g kidney wt⁻¹, P < 0.05) between 103 ± 2 and 81 ± 2 mmHg, producing an increase in the autoregulatory indices of RPF and GFR (Fig. 4, A and B). The additional infusion of furosemide with hypertonic albumin diminished autoregulation of RPF (3.55 ± 0.26 to 2.93 ± 0.23 ml·min⁻¹·g kidney wt⁻¹, P < 0.01) or GFR (1.12 ± 0.11 to 0.93 ± 0.08 ml·min⁻¹·g kidney wt⁻¹, P < 0.01) for a change in MAP of 102 ± 2 to 80 ± 1 mmHg, resulting in further elevations of autoregulatory indices of RPF and GFR (Fig. 4, A and B).

With administration of CPX, RPF (3.70 ± 0.27 to 3.29 ± 0.25 ml·min⁻¹·g kidney wt⁻¹, P < 0.05), and GFR (1.19 ± 0.11 to 1.06 ± 0.10 ml·min⁻¹·g kidney wt⁻¹, P < 0.05) similarly decreased following a change in MAP from 103 ± 2 to 82 ± 2 mmHg, resulting in an increase in the autoregulatory indices of RPF and GFR (Fig. 4, C and D). In contrast, under basal conditions, a decrease in MAP from 101 ± 3 to 78 ± 1 mmHg failed to alter both RPF (3.62 ± 0.29 to 3.58 ± 0.21 ml·min⁻¹·g kidney wt⁻¹) and GFR (1.06 ± 0.11 to 1.07 ± 0.12 ml·min⁻¹·g kidney wt⁻¹). As shown in Fig. 4, C and D, following addition of furosemide and hypertonic albumin, a decrease in MAP (101 ± 2 to 80 ± 1 mmHg) produced a further decline in autoregulation of RPF (3.62 ± 0.20 to 2.99 ± 0.19 ml·min⁻¹·g kidney wt⁻¹, P < 0.01) and GFR (1.11 ± 0.11 to 0.91 ± 0.06 ml·min⁻¹·g kidney wt⁻¹, P < 0.01), resulting in corresponding elevations of the autoregulatory indices for RPF and GFR (Fig. 4, C and D).

Finally, the combined effects of CPX and PPADS were assessed. Under basal conditions, RPF (3.57 ± 0.32 to 3.50 ± 0.26 ml·min⁻¹·g kidney wt⁻¹, n = 6) and GFR (0.99 ± 0.03 to 0.98 ± 0.03 ml·min⁻¹·g kidney wt⁻¹) showed good autoregulation between 102 ± 1 and 81 ± 2 mmHg. Combined treatment with CPX and PPADS diminished autoregulation of RPF (3.95 ± 0.29 to 3.28 ± 0.24 ml·min⁻¹·g kidney wt⁻¹, P < 0.01) and GFR (1.14 ± 0.02 to 0.95 ± 0.02 ml·min⁻¹·g kidney wt⁻¹, P < 0.01) for a change in MAP of 103 ± 2 to 82 ± 2 mmHg, resulting in marked increase in autoregulatory indices of RPF and GFR (Fig. 4, E and F). Additional infusion of furosemide and hypertonic albumin failed to produce any further change in autoregulation of RPF (3.52 ± 0.20 to 2.95 ± 0.17 ml·min⁻¹·g kidney wt⁻¹) or GFR (1.06 ± 0.03 to 0.90 ± 0.03 ml·min⁻¹·g kidney wt⁻¹) for a pressure decrease from 103 ± 2 to 83 ± 2 mmHg.

Effects of an angiotensin receptor blocker and combined treatment with Cx37,43GAP27 and Cx46GAP27 on renal autoregulation. Under control conditions, RPF (4.25 ± 0.17 to 4.23 ± 0.18 ml·min⁻¹·g kidney wt⁻¹, n = 6) and GFR (1.01 ± 0.06 to 1.02 ± 0.05 ml·min⁻¹·g kidney wt⁻¹) were well autoregulated in the range of MAP from 98 ± 2 to 82 ± 1 mmHg. Following combined intrarenal infusion of Cx37,43GAP27 and Cx46GAP27, autoregulation of RPF (2.73 ± 0.23 to 2.46 ± 0.19 ml·min⁻¹·g kidney wt⁻¹, P < 0.05) and GFR (0.90 ± 0.08 to 0.81 ± 0.08 ml·min⁻¹·g kidney wt⁻¹, P < 0.05) was impaired between 100 ± 1 and 81 ± 1 mmHg. Thus, the autoregulatory index to RPF (0.05 ± 0.04 to 0.52 ± 0.08, P < 0.05) and GFR (-0.04 ± 0.06 to 0.50 ± 0.07, P < 0.05) was increased by combined treatment with Cx37,43GAP27 and Cx46GAP27 (Fig. 5). Of interest, the autoregulatory index to RPF and GFR during combined treatment with Cx37,43GAP27 and Cx46GAP27 showed similar values as those of the respective GAP peptide, suggesting no additive effects. Full release of aortic clamp resulted in an elevation of blood pressure to 115 ± 3 mmHg. The administration of losartan caused a decline in blood pressure to 91 ± 2 mmHg. Subsequent reduction of renal arterial pressure to 79 ± 1 mmHg by

### Table 1. Effects of GAP peptide on RPF and GFR at basal pressure

<table>
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<tr>
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<th>Control</th>
<th>GAP</th>
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<tr>
<td><strong>RPF</strong></td>
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<tr>
<td>Cx37,43  (n = 12)</td>
<td>4.19±0.18</td>
<td>3.38±0.16*</td>
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<tr>
<td>Cx40 (n = 12)</td>
<td>4.48±0.21</td>
<td>3.74±0.18*</td>
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<tr>
<td>Cx43 (n = 12)</td>
<td>4.53±0.20</td>
<td>4.33±0.20</td>
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<tr>
<td><strong>GFR</strong></td>
<td></td>
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<tr>
<td>Cx37,43 (n = 12)</td>
<td>1.03±0.02</td>
<td>1.03±0.02</td>
</tr>
<tr>
<td>Cx40 (n = 12)</td>
<td>1.02±0.02</td>
<td>0.98±0.02</td>
</tr>
<tr>
<td>Cx43 (n = 12)</td>
<td>1.03±0.02</td>
<td>0.93±0.02*</td>
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RPF, renal plasma flow; GFR, glomerular filtration rate; Cx, connexin. *P < 0.05 from the respective control.
Fig. 4. PPADS, CPX, and furosemide with hyperoncotic albumin (FA) on autoregulation of renal plasma flow (A, C, E) and glomerular filtration rate (B, D, F).

* and #Significantly different from the value obtained in control and PPADS (or CPX) period, respectively.
the aortic clamp decreased RPF (3.77 ± 0.18 to 3.43 ± 0.15 ml min⁻¹ g kidney wt⁻¹, P < 0.05) and GFR (0.89 ± 0.05 to 0.82 ± 0.05 ml min⁻¹ g kidney wt⁻¹, P < 0.05). The addition of losartan to GAP peptides tended to increase autoregulatory index of RPF and GFR compared with GAP peptides alone, but statistical significance was not obtained (Fig. 5).

DISCUSSION

Using peptide antagonists against specific Cx subtypes, we have demonstrated for the first time that in vivo autoregulation of RPF and GFR in the whole kidney requires a contribution from gap junctional coupling involving Cx37 and Cx40 but not Cx43. Moreover, we have found that activation of both adenosine-1 and purinergic-2 receptors is necessary for autoregulation, but only the latter response requires gap junctional coupling. Our data are in line with our recent immunohistochemical studies, which have demonstrated expression of both Cx37 and Cx40 in the renin-secreting cells of the juxtaglomerular apparatus, and Cx40 in the extraglomerular mesangial cells, but an absence of Cx43 from these sites (39). These data also showed that all three Cxs were localized in preglobular endothelial cells, while only Cx37 was expressed in rat efferent arteriolar endothelial cells and that Cx43 appeared to be expressed in podocytes (39).

The GAP peptides used in this study did produce some impairment of renal autoregulation of either RPF or GFR, as measured by inulin. However, our previous study, in which GAP peptide-induced blood pressure increase was not controlled by preconstricting the clamp (39), indicated that the autoregulatory index of RPF (0.59 ± 0.08 for Cx37GAP27, 0.60 ± 0.07 for Cx40GAP27) and GFR (0.63 ± 0.06 for Cx37GAP27, 0.59 ± 0.06 for Cx40GAP27) did not differ from that of the present study. Intrarenal infusion of Cx40GAP27 inhibited renal autoregulation. Although this effect might be due to the intense expression of Cx40 in extraglomerular mesangial cells and inhibition of the TGF mechanism, it is also possible that Cx40GAP27 might directly inhibit the afferent arteriolar myogenic response, because Cx40 was also localized in the terminal afferent arteriolar myocytes. Indeed, Cx37,43GAP27 has been shown to inhibit the myogenic constriction of mesenteric artery, in which the vascular smooth muscle cells expressed Cx37 (6). Although the endothelium is not required for myogenic response of small renal arteries (20), ACh inhibits afferent arteriolar myogenic constriction possibly through the action of endothelium-derived hyperpolarizing factor (12). Thus, myoendothelial gap junctions may also modulate renal autoregulation. Myoendothelial gap junctions are present in the basilar and mesenteric arteries (9, 24). Interestingly, gap junctions linking the endothelium with the renin-secreting cells have been shown to contain Cx40 (10). Just (15) reported that myogenic response accounts for about 50% of renal autoregulation. However, it is likely that TGF mediates the major component of the renal autoregulation observed in the present study. Using micropuncture to interrupt TGF in a single nephron, Navar et al. (27) concluded that while there is a component mediating autoregulatory adjustments that is not governed by the TGF mechanism, it represents a relatively minor fraction at mean arterial pressures of about 100–110 mmHg. They estimated that the myogenic response contributes only 15% (at most) to the total autoregulatory tone. In harmony, our data showed that over 80% of autoregulation was
prevented during infusion of furosemide and hyperoncotic albumin, both of which inhibit TGF (26, 42). The present data are consistent with those of Peti-Peterdi (29) who reported that the spread of a TGF-induced calcium wave from the extraglomerular mesangial cells and renin-secreting cells to the afferent arteriole was blocked by an ATP scavenger enzyme cocktail, suramin, or nonselective gap junction blockers. Previous studies showed that activation of calcium channels underlies afferent arteriolar constriction by TGF (28, 38) and that heptanol inhibited TGF-induced afferent arteriolar constriction (30). The present results demonstrate for the first time that PPADS did not further worsen renal autoregulation when added with Cx40GAP27. Although one may expect that Cx40 could modulate the ultrafiltration coefficient from its location in the glomerulus, the present findings that Cx40GAP27 diminished renal autoregulation of RPF, as well as GFR, suggest that the site of action of Cx40GAP27 is more likely to be at the afferent arteriole. Taken together, these results suggest that gap junctions containing Cx40 in afferent arteriolar myocytes, including renin-secreting cells, or in the extraglomerular mesangium are involved in transducing purinergic signals mediating TGF.

Our data also show that renal autoregulation was impaired by Cx37,43GAP27. Because Cx43GAP26 did not alter renal autoregulation, we propose that Cx37, not Cx43, is involved in the regulation of TGF. Consistent with the data obtained with Cx40GAP27, PPADS did not further worsen renal autoregulation when added with Cx37,43GAP27. Collectively, these observations suggest that renin-secreting cells that express both Cx37 and Cx40 are involved in the pathway through which the purinergic signal of TGF is transduced. The absence of a cumulative effect of the combination of Cx40GAP27 with Cx37,43GAP27 makes it tempting to speculate that heteromeric connexons made of Cx37 and Cx40 form gap junctional hemichannels on renin-secreting cells, and these play an important role in mediating TGF. Alternatively, reduced upstream propagation of the TGF signal may contribute to a reduction in autoregulatory efficiency by the GAP peptide (33, 48). The TGF signal could be transduced from the renin-secreting cells through gap junctions, directly to afferent arteriolar myocytes, or indirectly via endothelial cells to arteriolar myocytes. Moreover, two connexins could transmit the TGF signal in series; that is, only Cx40GAP27 affecting intercellular signal transduction via extraglomerular mesangial cells. In addition, Cx hemichannels may be involved, although their existence is still not completely confirmed (32). GAP peptides may thus block Cx hemichannels on extraglomerular mesangial cells and inhibit ATP release, thereby preventing purinergic signals from reaching afferent arteriolar myocytes.

We have proposed that an important physiological mechanism like TGF should have redundancy, because TGF works in all nephrons despite nephron heterogeneity (42). Some nephrons have a small number of mesangial cells, which are positioned between the macula densa and afferent arteriole, but other nephrons assemble large numbers of mesangial cells in this position. In the latter nephrons, the passage from the macula densa to the afferent arteriole should result in the degradation of ATP into adenosine, thereby inducing afferent arteriolar constriction (35, 47). Alternatively, in the former nephrons, ATP could reach the afferent arteriole before degradation, again eliciting afferent arteriolar constriction (14). ATP-induced increase in cytosolic calcium or membrane depolarization in the extraglomerular mesangial cells may be transferred to afferent arteriolar myocytes through the renin-secreting cells (25, 29, 42). The present studies using CPX and PPADS provide the evidence that the full expression of TGF in vivo requires the activation of both adenosine-1 and purinergic-2 receptors in the whole kidney. Furthermore, our data show that blockade of adenosine, but not purinergic, receptors in combination with Cx40GAP27 or Cx37,43GAP27 produced further impairment of renal autoregulation, compared with GAP peptide alone, suggesting that adenosine participates in TGF independently of gap junctions formed by Cx37 and Cx40. In addition, the administration of losartan tended to elicit increases in autoregulatory indexes of RPF and GFR compared with Cx40GAP27 and Cx37,43GAP27 alone, presumably supporting an interaction of adenosine and ANG II in TGF (11, 34). However, the interpretation of the latter data requires caution, because the study had to be performed under rather low baseline blood pressures, and the autoregulatory stimulus induced by aortic clamping was therefore relatively small.

A caveat in regard to the interpretation of our results should be considered. Our recent data have shown that inhibition of Cx37 or Cx40 increases renin and ANG II (39), while other recent findings demonstrated that ANG II enhances afferent arteriolar constriction through adenosine (11, 34). In the present study, the administration of losartan elicited a large decrease in blood pressure and a reversal of renal vasoconstriction due to blockade of Cx37 and Cx40, supporting the notion that an inhibition of Cx37 and Cx40 activates the renin-angiotensin system. Recent data also indicate that Cx40 knock-out mice lack pressure-induced control of renin release (49). Further experiments with blockade of the renin-angiotensin system may be needed to assess precise roles of adenosine in renal autoregulation. The settings whereby arterial pressure can be controlled independently of the renin-angiotensin system may be assessed accordingly. Our recent data also demonstrated that renal autoregulation was preserved to 80 mmHg (42). Previous studies indicated that renal autoregulation was preserved down to 75 mmHg in young rats, but renal autoregulation disappeared below 95–100 mmHg in adult rats (2, 4, 37). The present observations that RPF and GFR were well autoregulated following pressure changes to 80 mmHg support the notion that age is an important determinant of autoregulatory range.

In summary, the present study has demonstrated for the first time that in vivo autoregulation of renal plasma flow and glomerular filtration rate in the whole kidney requires activation of both adenosine-1 and purinergic-2 receptors, and the latter response involves gap junctions comprising Cx37 and Cx40. The kidney plays an overdominant role in the development and maintenance of hypertension (28, 38) and the shift of renal autoregulation toward higher pressures underlies sodium-insensitive hypertension. Because Cxs control vascular tone and the expression of Cxs is altered in the hypertensive state (31), the present results suggest that future investigations of the pathophysiological roles of Cxs in the development of hypertension and the progression of chronic kidney diseases, including diabetes, are warranted.
animals. Parts of the data in this manuscript were presented in the Annual Meeting of American Society of Nephrology in San Diego, CA, in November 2006 and were published in abstract form.

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