Effects of Activation of Renal Adenosine A2 Receptor on Renal Function and Renin Release in Dogs

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ABSTRACT—Direct effects of adenosine A2 receptor activation on renal function were examined in dogs. When renal perfusion pressure was maintained constant at 100 mmHg, renal administration of a selective A2 receptor agonist, CGS 21680C (sodium salt of CGS 21680, 2-[*p*-(2-carboxyethyl) phenethylamino]-5′-*N*-ethylcarboxamido adenosine) (1 $\mu g \cdot kg^{-1} \cdot min^{-1}$), although it decreased blood pressure by 20 mmHg, increased renal blood flow and renin release, whereas glomerular filtration rate and urine flow were unaffected. These results suggest that stimulation of renal A2 receptor led to both afferent and efferent arteriolar dilatation, whereas renal A2 receptor plays a minor role in urine formation. CGS 21680C induced renin release and tachycardia that were blocked by propranolol, indicating these effects of A2 receptor stimulation appeared to be indirect.

Keywords: Adenosine, Adenosine A2 receptor, Renal hemodynamics

Renal adenosine plays an important role in the regulation of sodium homeostasis (1). Continuous infusion of adenosine into the renal artery causes a transient vasoconstriction followed by a prolonged vasodilation. These hemodynamic changes were accompanied by a fall in glomerular filtration rate (GFR) (2, 3). It has been proposed that adenosine constricts afferent arteriole and dilates efferent arteriole through the adenosine A1 and A2 receptor, respectively (4). Even though the importance of A1-receptor-mediated afferent arteriolar constriction in decreasing GFR has been well documented, the role of renal adenosine A2 receptor were controversial. Aki et al. (3) reported that intrarenal arterial infusion of adenosine decreased GFR by preferential dilatation of efferent arterioles, suggesting the importance of the A2 receptor. In contrast, administration of non-ionic contrast media to a conscious dog provoked simultaneous rises in renal plasma flow and GFR, both by 50%, that were completely blocked by adenosine A2 receptor antagonist (5). Such results are strongly indicative of preferential afferent arteriolar dilatation by A2 receptor stimulation. Furthermore, the role of A2 receptor in urine formation have not been well characterized. After the development of a specific A2 receptor agonist, CGS 21680 (6), Levens et al. first examined the effects of intravenous infusion of CGS 21680A (7). Although consistent renal vasodilatation was accompanied by some degree of changes in GFR, urine formation and renin release, simultaneous hypotension induced by the agonist made the interpretation of the results complicated. They repeated the study by direct intrarenal arterial infusion of CGS 21680A, while taking care not to reduce systemic blood pressure by restricting the dose used (8). However, with such restriction of the dose, results may not necessarily reveal full manifestation of renal A2 receptor activation. Therefore, in order to further define the role of the renal A2 receptor, we administered a hypotensive dose of CGS 21680 directly into the renal artery of anesthetized dogs while keeping the renal perfusion pressure constant with an adjustable aortic clamp; thereby, the direct action of the agonist was elucidated. CGS 21680A is the hydrochloride salt and CGS 21680C is the sodium salt of CGS 21680, 2-[*p*-(2-carboxyethyl) phenethylamino]-5′-*N*-ethylcarboxamido adenosine. We used CGS 21680C for A2 receptor activation.

Adult mongrel dogs anesthetized with intravenous pentobarbital sodium (30 mg/kg) were surgically prepared for renal function monitoring. Preliminary studies using dogs indicated that intrarenal arterial infusion of CGS 21680C at a rate of 1 $\mu g \cdot kg^{-1} \cdot min^{-1}$ decreased blood pressure by approx. 25 mmHg and increased renal blood flow by 25% with an increase in renal venous plasma re-

nin activity. To examine the direct action of CGS 21680C on renal hemodynamics, urine formation and renin release, anesthetized dogs were uninephrectomized. Renal blood flow of the remaining kidney was measured by an electromagmetic flowmeter through a flow probe (MFV-2100; Nihon Kohden, Tokyo). All visible renal nerves around the renal artery were surgically dissected to examine the direct action of CGS 21680C on the kidney. The ureter was cannulated for timed urine collection. After a control clearance period, a second clearance was performed while the renal perfusion pressure was lowered and maintained at 100 mmHg by an adjustable aortic clamp placed just above the origin of the renal artery of the remaining kidney. Then CGS 21680C was infused into the renal artery at a rate of 1 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 20 min. During infusion, two clearances were made at 5 and 15 min while keeping the renal perfusion pressure about 100 mmHg. Twenty minutes after the cessation of CGS 21680C, an additional clearance period was followed by the release of aortic clamp and then the last clearance was performed. Systemic blood pressure was monitored through a catheter whose tip was placed in the thoracic aorta, and renal perfusion pressure was monitored in the abdominal aorta below the origin of the renal artery.

Figure 1 shows the effects of CGS 21680C on renal and systemic hemodynamics, tubular function and renin release (n=5). With infusion of CGS 21680C, systemic blood pressure decreased by approx. 20 mmHg with an increase in heart rate. Renal blood flow increased from 144±13 to 194±16 ml/min while renal perfusion pressure remained constant. Even with this marked renal vasodilation, GFR was maintained at a constant level. These results strongly suggested that both afferent arterioles and efferent arterioles were dilated with A2 receptor stimulation. It was established that renal blood flow and GFR were well autoregulated when renal perfusion pressure was reduced down to 100 mmHg while renal blood flow but not GFR remained constant down to 70 mmHg (autoregulatory limit) in anesthetized dogs (9). In fact, the former notion was also supported by the present finding that reduction of renal perfusion pressure to 100 mmHg did not affect either renal blood flow or GFR (Fig. 1). The reduction of renal perfusion pressure in this range causes preferential afferent arteriolar dilation (9). Even with such a pre-existing dilated tone of the afferent arteriole, CGS 21680C elicited renal vasodilatation without any changes in GFR, indicating that stimulation of A2 receptor indeed potently dilates not only efferent arterioles but also afferent arterioles. This is in marked contrast to the finding that adenosine preferentially dilated the efferent arteriole and reduced GFR (3). Such a difference was obviously derived from the differences of adenosine and CGS 21680C in terms of the selectivity of adenosine receptor subtypes. Adenosine acts at both A1 and A2 receptor, whereas CGS 21680C selectively acts at the A2 receptor. Therefore, differential effects on GFR could be explained as follows: Although adenosine elicited both afferent and efferent arteriolar dilation via A2 receptors, simultaneous constriction of afferent arterioles via A1 receptor converted the afferent arteriolar dilatation to constriction, thereby reducing GFR. Furthermore, the remaining efferent arteriolar dilatation synergistically acted to decrease GFR.

In the presence of marked renal vasodilation observed by A2 receptor stimulation, urine flow or urinary excretion of sodium and potassium were not affected, suggesting that the adenosine A2 receptor does not appear to play an important role in the tubular reabsorption in vivo. Although a recent study using anesthetized rats suggested that A2 agonist directly stimulated tubular sodium reabsorption (10), concomitant hypotension and fall in GFR made the interpretation of their results difficult.

With infusion of CGS 21680C, renin release was markedly stimulated. Churchill et al. (11) reported that intravenous administration of a relatively specific A2 receptor agonist, 5′-*N*-ethylcarboxamide adenosine, to rats increased plasma renin concentration, while administration of an A1 receptor agonist decreased it even when these agonists were equally hypotensive. Although the authors suggested that activation of A2 receptor stimulated renin release, it was unknown whether such an action was direct or indirect. Since renal perfusion pressure was maintained constant in the present study, renal baroreceptor-mediated renin release (12) is unlikely. In as much as CGS 21680C elicited a fall in systemic blood pressure, reflex-activation of the sympathetic nervous system may have stimulated the renin release. This hypothesis was tested by using a β-adrenergic receptor antagonist. Following treatment of dogs with propranolol (0.3 $mg \cdot kg^{-1} \cdot min^{-1}$ after bolus injection of 0.6 mg/kg, i.v.), a similar experiment was performed as described above. This dose of propranolol has been proven to block the decrease in blood pressure and the increase in heart rate elicited by intravenous isoproterenol (0.5 μg/kg, i.v.); control vs propranolol, −39±3.1 vs −8.7±2.2 mmHg, +87±14 vs +17±7 beats/min (n=3) ($P<0.05$, paired *t*-test). With propranolol, although systemic and renal hemodynamic responses to CGS 21680C were essentially the same as those seen without propranolol (data not shown), stimulatory action of CGS 21680C on renin release was significantly attenuated (Fig. 2, n=7). In addition, CGS 21680C-induced tachycardia (increase by 31.0±7.0 beats/min) was markedly attenuated when dogs were pretreated with propranolol (increase by 13.1±2.9 beats/min). Although circulating catecholamine concen-

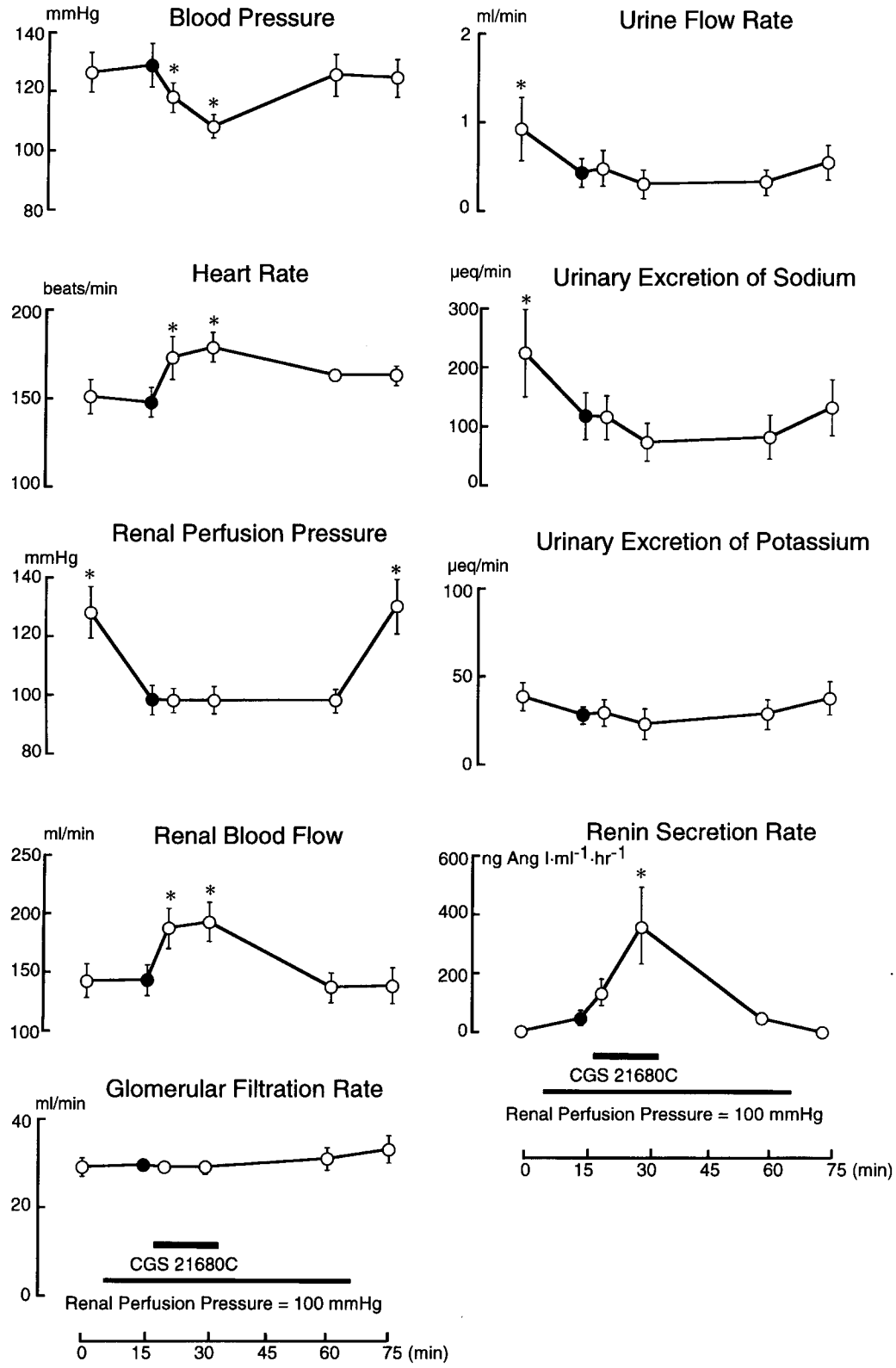

Fig. 1. Effects of renal administration of CGS 21680C on systemic and renal hemodynamics, urine formation and renin release at constant renal perfusion pressure. CGS 21680C was infused into the renal artery at a rate of 1 $\mu g \cdot kg^{-1} \cdot min^{-1}$ while renal perfusion pressure was maintained constant. Data are reported as the mean ± S.E.M. *$P < 0.05$, compared to the second clearance period when renal perfusion pressure was fixed at 100 mmHg (closed circle).

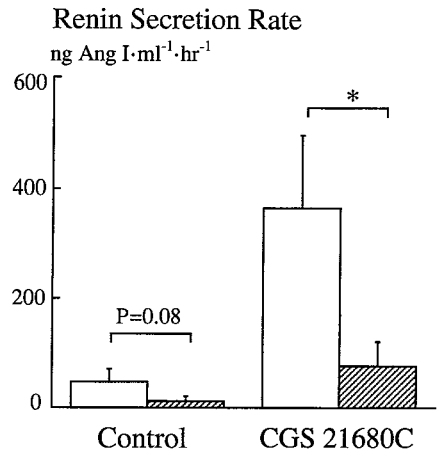

Fig. 2. Effects of propranolol on CGS 21680C-induced increase in renin secretion rate. Open columns indicate data without propranolol and hatched columns indicate data with propranolol. Data are reported as the mean±S.E.M. *$P<0.05$.

tration was not determined in the present experiment, these results indicate that stimulated renin release elicited by CGS 21680C was due to reflex-mediated β-adrenergic stimulation. Since renal nerves were surgically dissected, an increase in circulating catecholamine from the adrenal glands may likely be involved. Furthermore, the fact that CGS 21680C-induced tachycardia was blocked by propranolol treatment suggests that CGS 21680C-induced tachycardia was due to reflex-mediated sympathetic nervous activation but not due to direct activation of A2 receptors in the heart.

In summary, the present experiments suggested that stimulation of renal adenosine A2 receptor was capable of dilating both afferent and efferent arterioles. Adenosine A2 receptor does not appear to be involved in tubular reabsorption of electrolytes and water. The stimulated renin release and tachycardia observed during A2 receptor activation were most likely due to baroreflex-mediated sympathetic nervous activation.

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