Effects of KB-2796, a New Diphenylpiperazine Calcium Antagonist, on Renal Hemodynamics and Urine Formation in Anesthetized Dogs

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ABSTRACT — The effects of KB-2796, a new calcium antagonist with a diphenylpiperazine moiety, on renal hemodynamics and urine formation were investigated in anesthetized dogs. Intravenous infusion of KB-2796 (10, 30, and 100 μg/kg/min) decreased mean blood pressure (MBP) and renal vascular resistance (RVR) in a dose-dependent manner, but did not change renal blood flow (RBF). At the highest dose, glomerular filtration rate (GFR) and urine flow (UF) tended to decrease. Nicardipine (0.1, 0.3, and 1 μg/kg/min) also dose-dependently decreased MBP, RVR, GFR, and UF. When KB-2796 was infused into the renal artery at lower doses of 3 and 10 μg/kg/min, UF and urinary excretion of electrolytes increased without a significant change in RBF and GFR. Intrarenal infusion of KB-2796 at 30 μg/kg/min and nicardipine at 0.3 μg/kg/min produced a significant increase in GFR, RBF, UF, urinary excretion of electrolytes, and renin secretion rate. These results suggest that KB-2796 administered intrarenally exerts a diuretic action via tubular effects and the alteration of renal hemodynamics. However, its diuretic action might be masked by diminished urine formation via a reflex activation of the sympathetic nerves and/or via a reduction of renal perfusion pressure when it is administered systemically.

Keywords: KB-2796, Calcium antagonist, Renal hemodynamics, Urine formation, Plasma renin activity

KB-2796, 1-[bis(4-fluorophenyl)methyl]-4-(2,3,4-trimethoxybenzyl)piperazine dihydrochloride, is a newly synthesized cerebral vasodilator (1, 2) which has been shown to block the voltage-dependent calcium channel (3, 4). KB-2796 has been reported to selectively inhibit the contraction of cerebral arteries induced by K^+ and prostaglandin $F_{2\alpha}$ (5) and to preferentially increase cerebral blood flow in anesthetized dogs (6).

The calcium antagonists are compounds of extremely diverse structure (7). The 1,4-dihydropyridine derivatives nifedipine, nicardipine, and nitrendipine, and the benzothiazepine derivative diltiazem are now widely used in patients with hypertension, angina pectoris, and/or cerebrovascular disease. The effects of these calcium antagonists on renal hemodynamics and function are generally well-known in man (8) and animals (9–13). However, the diphenylpiperazine derivatives KB-2796, flunarizine, and cinnarizine, which are used as cerebral vasodilators (14, 15), have not yet been studied well enough to characterize their renal effects.

The present study was designed to elucidate the direct and systemic effects of KB-2796, in comparison with those of nicardipine, a cerebral vasodilator of the 1,4-dihydropyridine calcium antagonist group, on renal hemodynamics and urine formation in anesthetized dogs.

MATERIALS AND METHODS

Drugs

KB-2796 was synthesized in the New Drug Research Laboratories, Kanebo, Ltd., Osaka, Japan (1). Nicardipine was purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Both drugs were dissolved in 0.9% saline for intravenous and intrarenal administration.

General procedure

Mongrel male or female dogs (9 – 18 kg) were anesthetized with sodium pentobarbital (30 mg/kg, i.v.) and were then intubated and artificially ventilated on room air with a respirator. The right brachial vein and artery were catheterized for infusion of a creatinine solution and for arterial blood sampling, respectively. A catheter was also placed in the abdominal aorta via the right femoral artery, and systemic blood pressure was continuously monitored with a pressure transducer (MPU-0.5, Nihon Kohden, Tokyo, Japan). The left kidney was exposed through a retroperitoneal flank incision and the renal artery was dissected free from surrounding tissue. An electromagnetic flow probe (2.5 – 3.5 mm in diameter, FB-type, Nihon Kohden) was attached at the renal artery to measure renal blood flow (RBF) with a square-wave flowmeter (MFV-2100, Nihon Kohden). The left ureter was cannulated for the collection of timed urine samples. For intravenous administration, a polyethylene tube was connected to the right brachial vein catheter to infuse 0.9% saline or drug solutions at the rate of 0.5 ml/min. For intrarenal administration, the renal artery was denervated by stripping all visible nerve fibers followed by application of 5% phenol in 70% ethanol. A curved 23-gauge needle connected to a polyethylene tube was then inserted into the left renal artery proximal to the flow probe for infusion of 0.9% saline or drug solutions at a rate of 0.5 ml/min. In some dogs, another curved 18-gauge needle was inserted into the left renal vein for the collection of renal venous blood samples. After completion of surgery, a priming dose of creatinine (100 mg/kg) was given, followed by a sustaining infusion of 0.9% saline containing creatinine (50 mg/kg/min) at the rate of 2.0 ml/min. A 60- to 90-min period was allowed for stabilization of systemic blood pressure, RBF, and urine flow (UF) before experiments.

After all measurements were completed, the left kidney was removed, stripped of all surrounding tissue, blotted on filter papers, and weighed.

Experimental protocol 1: intravenous administration

The experiment consisted of a control period, three consecutive drug infusion periods, and a recovery period. During the control period, vehicle (0.9% saline) was first infused into the right brachial vein for 20 min. Subsequently, drug solutions at three successive doses were cumulatively infused at 20-min intervals for 60 min. After the infusion of drug, vehicle was again infused for 20 min. Urine collection was performed every 10 min. At the midpoint of each urine collection period, a systemic arterial blood sample was obtained from the right brachial artery, and plasma was immediately separated by centrifugation. The urine and plasma samples were analyzed for creatinine and electrolytes.

Experimental protocol 2: intrarenal administration

The experiment consisted of control, drug infusion, and recovery periods. During the control period, vehicle was first infused into the left renal artery for 20 min, to be followed by replacement with drug solution for 20 min. Subsequently, vehicle was again infused for 30 min. Urine was collected every 10 min. At the midpoint of each urine collection period, systemic arterial and renal venous blood samples were simultaneously obtained from the right brachial artery and left renal vein, respectively. The urine and plasma samples were analyzed for creatinine, electrolytes, and plasma renin activity (PRA).

Analytical procedures

Sodium and potassium were measured by flame photometry (205D, Hitachi, Tokyo, Japan), and chloride was analyzed with a chloridemeter (4-2500, Buchler, Fort Lee, NJ, U.S.A.). Creatinine was determined by colorimetry according to the Jaffe reaction (16), and glomerular filtration rate (GFR) was estimated by creatinine clearance. PRA was determined by radioimmunoassay of angiotensin I (AI) and expressed as nanograms of AI per milliliter during 1 hr of incubation (17). Renin secretion rate (RSR) was calculated as the product of renal plasma flow and the difference between renal venous PRA and arterial PRA (10). Renal vascular resistance (RVR) was calculated as RVR = MBP/RBF where MBP is the mean blood pressure.

All data except for findings with MBP and PRA are expressed as units per gram of kidney weight.

Statistical analyses

All values are shown as the mean ± S.E.M. The data were analyzed by two-way analysis of variance with complete randomized block, and significant differences were determined by the least significant difference test (18). P values less than 0.05 were considered to indicate a significant difference.

RESULTS

Effects of intravenous infusion of KB-2796 and nicardipine on renal hemodynamics and urine formation in anesthetized dogs

Intravenous infusion of KB-2796 at three successive doses of 10, 30, and 100 μg/kg/min did not affect RBF. The calculated RVR decreased dose-dependently from the control value of 40.4 ± 3.5 to the minimum

value of 31.1 ± 4.4 mmHg/ml/g·min ($P < 0.05$), with a concomitant reduction of MBP. GFR slightly decreased at higher doses of 30 and 100 μg/kg/min. UF and urinary excretion of sodium, potassium, and chloride ($U_{Na}V$, U_KV, and $U_{Cl}V$) showed a tendency toward decrease as doses increased, but these changes were not statistically significant (Fig. 1).

Intravenous infusion of nicardipine at three successive doses of 0.1, 0.3, and 1 μg/kg/min also produced a dose-dependent and significant decrease in RVR and MBP, but RBF did not change significantly. At a dose of 0.3 μg/kg/min, UF and urinary excretion of electrolytes showed a tendency toward decrease, and GFR significantly decreased. When nicardipine was infused at the highest dose of 1 μg/kg/min, GFR, UF, $U_{Na}V$, and $U_{Cl}V$ significantly decreased, and the changes in UF and $U_{Na}V$ lasted over 20 min after the cessation of its infusion (Fig. 2).

Effects of intrarenal infusion of KB-2796 and nicardipine on renal hemodynamics and urine formation in anesthetized dogs

As shown in Fig. 3, intrarenal infusion of KB-2796 at the lowest dose of 3 μg/kg/min caused a significant increase in UF and urinary excretion of electrolytes without significantly affecting RBF, RVR, and GFR. Infusion of KB-2796 at 10 μg/kg/min also increased UF and urinary excretion of electrolytes; UF increased by 326% compared with the control value. RBF also increased by 27%, but this change was not statistically significant. No significant changes in MBP, RVR, and GFR were observed with this dose of KB-2796. When KB-2796 was infused at the highest dose of 30 μg/kg/min, a marked and significant increase in UF and urinary excretion of electrolytes was observed; UF increased by 340%. RBF and GFR also significantly increased by 35% and 28%, respectively. All variables returned gradually to the respective control level after the cessation of KB-2796.

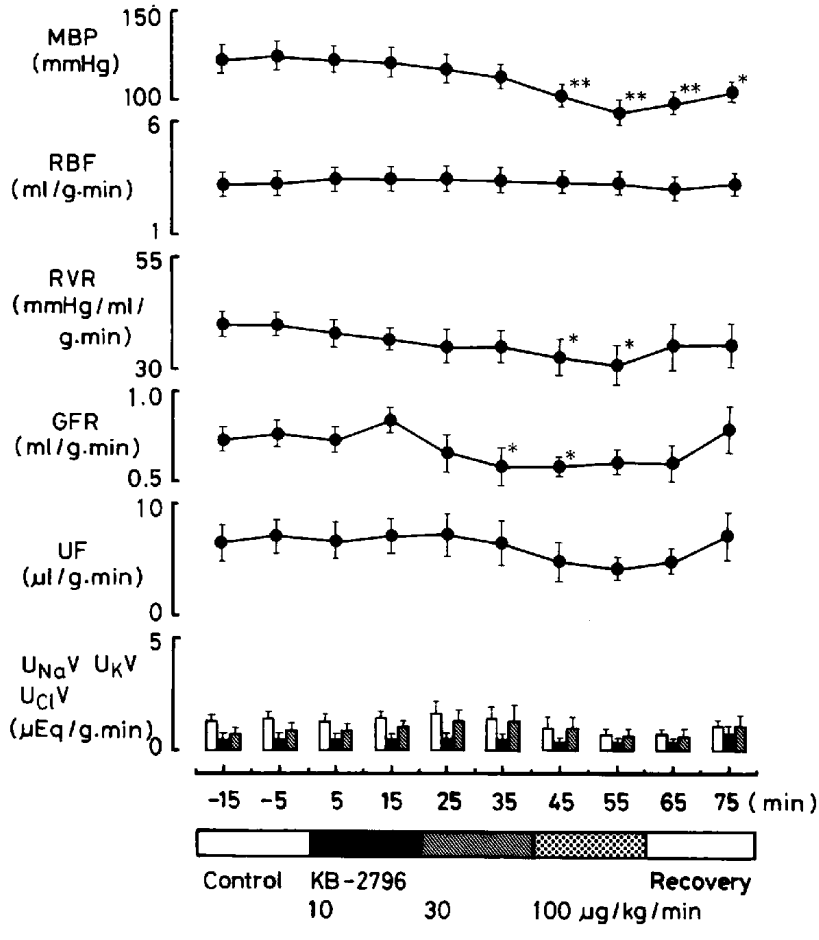

Fig. 1. Effects of intravenous infusion of KB-2796 on renal hemodynamics and urine formation in anesthetized dogs. Each value represents the mean ± S.E.M. of five dogs. □: $U_{Na}V$, ■: U_KV, ▨: $U_{Cl}V$, g = grams of kidney weight. $^*P < 0.05$, $^{**}P < 0.01$, compared with the values observed at −5 min.

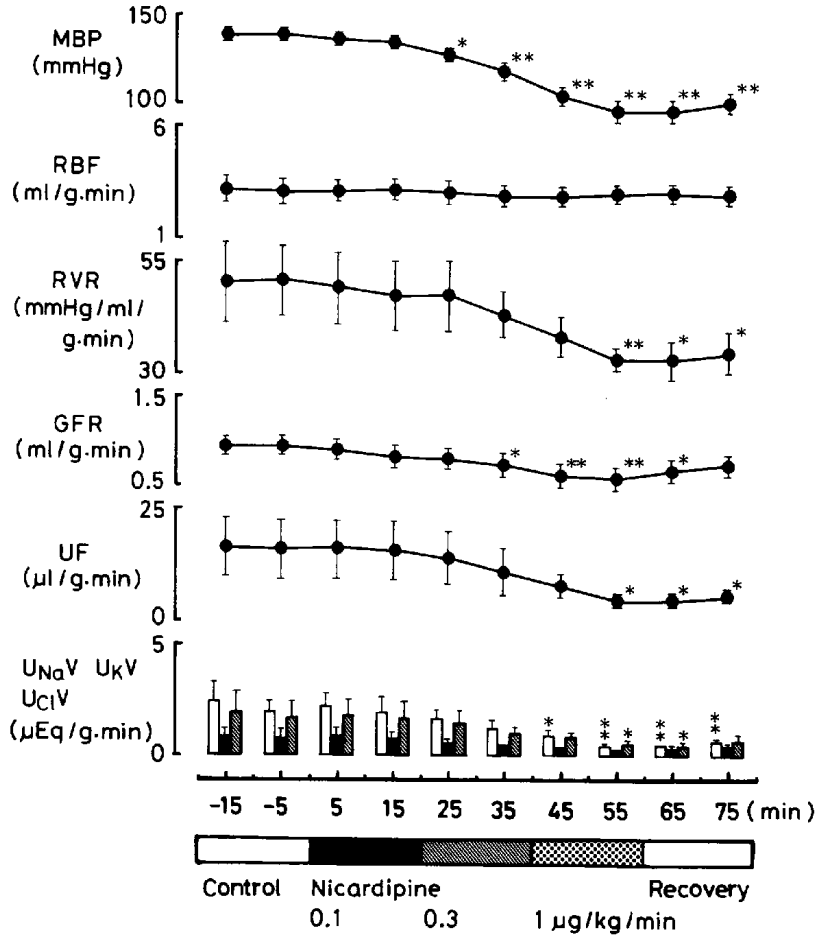

Fig. 2. Effects of intravenous infusion of nicardipine on renal hemodynamics and urine formation in anesthetized dogs. Each value represents the mean ± S.E.M. of five dogs. □: $U_{Na}V$, ■: U_KV, ▨: $U_{Cl}V$, g = grams of kidney weight. $^*P < 0.05$, $^{**}P < 0.01$, compared with the values observed at −5 min.

Intrarenal infusion of nicardipine at doses of 0.1 and 0.3 μg/kg/min also elicited a significant increase in RBF, GFR, UF, and urinary excretion of electrolytes, and a significant decrease in RVR. The maximum changes in RBF, GFR, and UF were 29%, 16%, and 282%, respectively, at the dose of 0.1 μg/kg/min and were 34%, 16%, and 401%, respectively, at the dose of 0.3 μg/kg/min. No significant change in MBP was observed with both doses of nicardipine (Fig. 4).

Effects of intrarenal infusion of KB-2796 and nicardipine on PRA and RSR in anesthetized dogs

When KB-2796 at 10 and 30 μg/kg/min and nicardipine at 0.3 μg/kg/min were infused into the renal artery, arterial PRA and venous PRA showed a tendency toward increase. The calculated RSR also significantly increased to 5.5-fold and 6.8-fold of each control value during infusion of KB-2796 (30 μg/kg/min) and nicardipine (0.3 μg/kg/min), respectively (Table 1).

DISCUSSION

Intravenous infusion of KB-2796 (10, 30, and 100 μg/kg/min) to anesthetized dogs produced a significant reduction in RVR, with a concomitant decrease of MBP, thus indicating significant renal vasodilation. However, RBF did not change. Similar results were also obtained by intravenous infusion of nicardipine (0.1, 0.3, and 1 μg/kg/min). We have previously reported that KB-2796 inhibited high KCl-induced contraction, high KCl-induced ^{45}Ca influx, and [^{3}H]-nitrendipine binding in dog vascular smooth muscle at similar concentrations, indicating that KB-2796 inhibits calcium entry through the L-type calcium channel (4). It is conceivable that such a calcium channel antagonism may contribute to the renal vasodilation induced by intravenous infusion of KB-2796. However, the kidney has been known to have an intrinsic function of maintaining RBF constantly in spite of changes in perfusion pressure (19). Therefore, the renal vasodila-

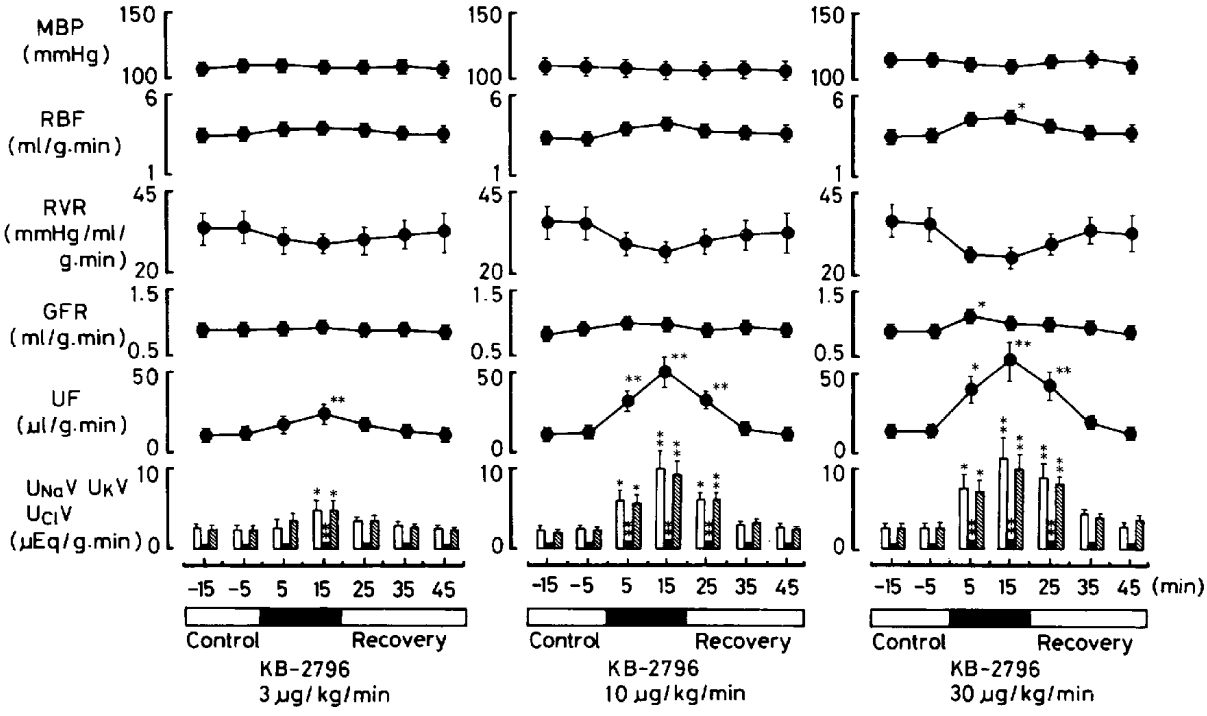

Fig. 3. Effects of intrarenal infusion of KB-2796 on renal hemodynamics and urine formation in anesthetized dogs. Each value represents the mean ± S.E.M. of five to seven dogs. □: $U_{Na}V$, ■: U_KV, ▨: $U_{Cl}V$, g = grams of kidney weight. $^*P < 0.05$, $^{**}P < 0.01$, compared with the values observed at −5 min.

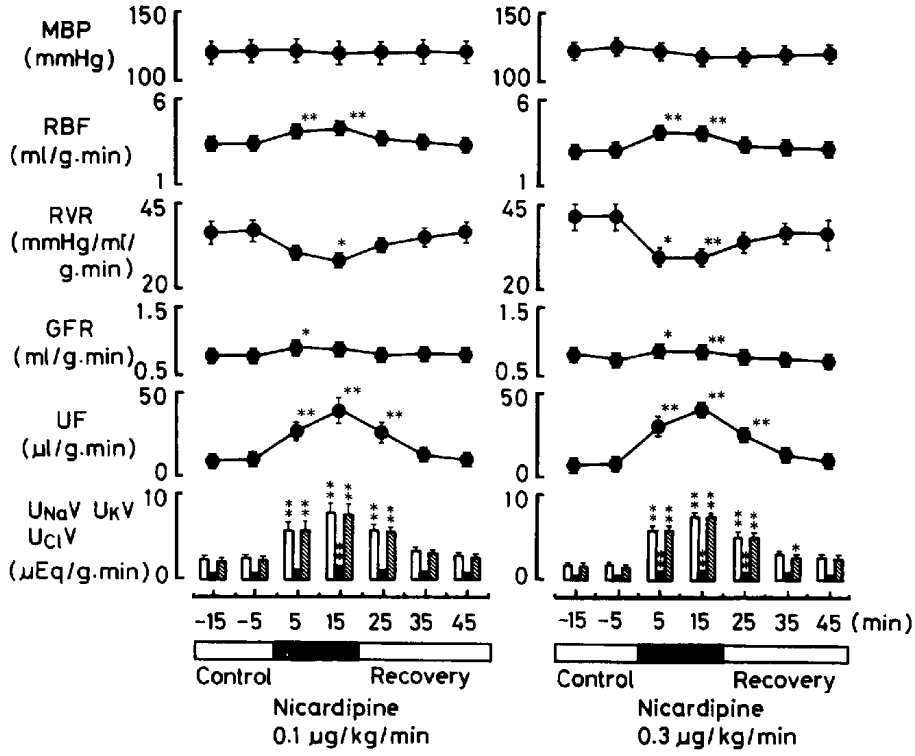

Fig. 4. Effects of intrarenal infusion of nicardipine on renal hemodynamics and urine formation in anesthetized dogs. Each value represents the mean ± S.E.M. of five or six dogs. □: $U_{Na}V$, ■: U_KV, ▨: $U_{Cl}V$, g = grams of kidney weight. $^*P < 0.05$, $^{**}P < 0.01$, compared with the values observed at −5 min.

Table 1. Effects of KB-2796 and nicardipine on plasma renin activity (PRA) and renin secretion rate (RSR) in anesthetized dogs

Periods	Arterial PRA	Venous PRA	RSR
	(ng AI/ml/hr)		(ng AI/hr/g·min)
Control	1.78 ± 1.1	1.98 ± 1.3	0.34 ± 0.3
KB-2796 (10 μg/kg/min)	2.27 ± 1.2	2.63 ± 1.4	0.84 ± 0.3
Recovery	2.00 ± 1.0	2.36 ± 1.4	0.54 ± 0.5
Control	1.54 ± 0.7	2.06 ± 1.0	0.79 ± 0.3
KB-2796 (30 μg/kg/min)	4.40 ± 1.0	6.30 ± 1.4*	4.37 ± 1.5*
Recovery	3.24 ± 0.7	3.86 ± 0.7	1.11 ± 0.4
Control	2.54 ± 0.4	2.76 ± 0.5	0.37 ± 0.1
Nicardipine (0.3 μg/kg/min)	7.72 ± 2.0	8.80 ± 1.9	2.51 ± 0.4**
Recovery	7.04 ± 3.0	7.23 ± 3.2	0.36 ± 0.3

Each value represents the mean ± S.E.M. obtained from five dogs. The control, drug infusion and recovery periods indicate the points of −5, 15 and 35 (or 45) min, respectively, described in Figs. 1 and 2. *$P < 0.05$, **$P < 0.01$, compared with the control.

tion observed during intravenous infusion of KB-2796 may be due to autoregulation of RBF, because MBP simultaneously decreased.

Previously, Kanazawa et al. (6) reported that bolus intravenous injection of KB-2796 and nicardipine produced a transient decrease in RBF, followed by a subsequent increase in anesthetized dogs. The subsequent increase in RBF was observed after recovery of the hypotensive response. In the present study, however, intravenous infusion of both drugs did not affect RBF. These inconsistent changes in RBF may have resulted from differences in the method of drug administration (bolus injection and infusion) and sympathetic reflex tone under the experimental conditions.

Intravenous infusion of KB-2796 at the highest dose of 100 μg/kg/min tended to decrease GFR, UF, and urinary excretion of electrolytes, with a marked reduction of MBP. Similar results were obtained by nicardipine, but at the highest dose of 1 μg/kg/min, it caused more pronounced reduction in these parameters than KB-2796. In general, marked hypotension elicited by peripheral vasodilators is accompanied by diminished urine formation via a reflex activation of the sympathetic nerves and/or via a decrease in renal perfusion pressure (20–22). The modest antidiuretic actions induced by KB-2796 and nicardipine are also assumed to be due to these factors.

Most calcium antagonists have been shown to exert a diuretic effect as a direct action on the kidney (9–11, 13). In the next experiment, we investigated the direct renal actions of KB-2796. KB-2796 was infused into the renal artery in the denervated kidney, to avoid extrarenal effects and the influence of renal nerves in the response to the drug. We found that intrarenal infusion of KB-2796 at the lower doses of 3 and 10 μg/kg/min produced a increase in UF and urinary excretion of electrolytes without a significant change in RBF and GFR. This result suggests that KB-2796 may produce a diuretic effect through a direct inhibitory action on the renal tubular reabsorption of sodium and water. On the other hand, intrarenal infusion of KB-2796 at the highest dose of 30 μg/kg/min caused a marked diuresis with a significant increase in RBF and GFR. Similar results were obtained by intrarenal infusion of nicardipine (0.1 and 0.3 μg/kg/min), as observed by Abe et al. (10). Earley and Friedler (23) have indicated that the increase in RBF causes diuresis as a consequence of the washout of medullary solutes by increased medullary blood flow. Thus, KB-2796 at higher doses seems to exert additional diuretic action that is induced by a renal vasodilation and an increment of GFR. Previously, nicardipine has been reported to produce diuresis via alteration of renal hemodynamics and/or via a direct inhibitory effect on sodium reabsorption in the proximal tubule (10). Taken together, it seems that the changes in renal hemodynamics and urine formation induced by KB-2796 are qualitatively similar to those seen with nicardipine, and these effects appear to be mediated by calcium channel antagonism.

Several vasodilating agents have already been shown to increase renin release from the kidney (24–26). In the present study, intrarenal infusion of KB-2796 (30 μg/kg/min) increased PRA and RSR. Intrarenal infusion of nicardipine (0.3 μg/kg/min) also produced similar responses in PRA and RSR, but this change in RSR was more prominent than that induced by KB-2796. Renin release is increased by various factors, e.g., stimulation of the baroreceptor via vasodilation and re-

duction of blood pressure in the renal artery, stimulation of the macula densa via increase of the distal tubular load, and decrease of intracellular calcium in the juxtaglomerular cells (27–29). It is likely that KB-2796 inhibits calcium influx into the juxtaglomerular cells and therefore promotes renin release. However, because KB-2796 also affected various other parameters related to the mechanism of renin release, it may be assumed that multiple factors are involved in the increase of renin release that was induced by KB-2796 in the present study.

In summary, our results indicate that KB-2796 exerts a diuresis, via tubular effects and the alteration of renal hemodynamics, by direct action on the kidney. However, its diuretic action might be masked by diminished urine formation via a reflex activation of the sympathetic nerves and/or via a reduction of renal perfusion pressure when it is administered systemically.

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