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# Prevention of cyclosporine A-induced renal vasoconstriction by the endothelin receptor antagonist SB 209670

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**Abstract**

Administration of endothelin to inactin-anesthetized rats resulted in a significant renal vasoconstriction as evidenced by a reduction in both renal plasma flow and glomerular filtration rate. Infusion of the novel nonpeptide endothelin $ET_A/ET_B$ receptor antagonist, (±)-SB 209670, [(1*RS*-2*SR*,3*RS*)-3-(2-carboxymethoxy-4-methoxy-phenyl)-5-(prop-1-yloxy)indane-2-carboxylic acid], significantly attenuated the renal vascular effects of endothelin-1. Intravenous administration of cyclosporine A (50 mg/kg) caused a significant reduction in renal plasma flow and glomerular filtration rate and urine flow and a dramatic increase in renal vascular resistance. Concomitant infusion of (±)-SB 209670 abolished the cyclosporine A-induced reduction in renal plasma flow and glomerular filtration rate and attenuated the cyclosporine A-induced fluid retention. The data indicate that endothelin is involved in the acute renal effects of cyclosporine A.

*Keywords:* Cyclosporine A; Endothelin; Nephrotoxicity; Renal failure

## 1. Introduction

Cyclosporine A is a potent immunosuppressive agent that is widely used, however, it has a number of side effects including nephrotoxicity. The mechanism for the nephrotoxicity is unclear but may involve prolonged renal vasoconstriction. Evidence is growing that endothelin mediates cyclosporine A-induced vasoconstriction and subsequent nephrotoxicity. Thus, cyclosporine A causes endothelin release from endothelial cells (Bunchman and Brookshire, 1991) and renal tubular and mesangial cells (Nakahama, 1990; Moutabarrik et al., 1991). In addition, there is an upregulation of renal endothelin receptors in rats with cyclosporine A-induced nephrotoxicity (Nambi et al., 1990; Awazu et al., 1991) and a recent study on mesangial cells in culture indicated that cyclosporine A leads to an increased expression of endothelin $ET_B$ receptor mRNA (Takeda et al., 1994). In addition, cyclosporine appears to increase the renal production of endothelin as evidenced by an increased urinary excretion (Brooks et al., 1991; Benigni et al., 1991). Treatment with nifedipine can attenuate both the cyclosporine A-induced renal dysfunction and the increased urinary endothelin excretion (Brooks et al., 1991). (±)-SB 209670 is a potent nonpeptide endothelin $ET_A/ET_B$ receptor antagonist that is effective in animal models of ischemia-induced acute renal failure (Brooks et al., 1994b; Gellai et al., 1995) and radiocontrast nephrotoxicity (Brooks and DePalma, 1995). In the present study, we have therefore evaluated the effect of (±)-SB 209670 on the acute vasoconstrictor activity of cyclosporine A in rats.

## 2. Materials and methods

### 2.1. Animal preparation

Male Sprague-Dawley rats weighing approximately 300 g were anesthetized with inactin (100 mg/kg, i.p.). A tube was placed in the trachea to aid respiration, and the femoral vein and the femoral artery catheterized for i.v. infusion and for measuring arterial blood

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pressure, respectively. In addition, a catheter was placed in the bladder via a suprapubic incision in order to collect urine.

### 2.2. Experimental protocols

Following surgery, rats received 2 ml of donor rat plasma intravenously and were then primed with 2 ml of Ringer's solution containing [$^{14}$C]inulin (approximately 3000 cpm/50 $\mu$l) and [$^{3}$H]*p*-aminohippuric acid (approximately 33 000 cpm/50 $\mu$l) followed by an infusion of the same solution at a rate of 55 $\mu$l/min.

Initial studies were performed to evaluate the effect of (±)-SB 209670, [(1*RS*-2*SR*,3*RS*)-3-(2-carboxymethoxy-4-methoxy-phenyl)-5-(prop-1-yloxy)indane-2-carboxylic acid], on the renal vasoconstrictor effects of endothelin-1. 60 min following the initiation of infusion of clearance markers, a 30 min urine collection was made, with a blood sample (200 $\mu$l) taken at the end. Immediately following collection of urine, an infusion of (±)-SB 209670 (30 $\mu$g/kg · min) or vehicle was initiated. 5 min later, endothelin-1 (100 pmol/kg) or vehicle was injected i.v. 5 min later, a second 30 min urine collection period was obtained, with a blood sample taken at the end. Blood samples were centrifuged and 50 $\mu$l samples of plasma and urine were placed in 10 ml of liquid scintillation fluid and counted in a liquid scintillation counter. Four groups of animals were used, animals that received vehicle throughout ($n = 9$), animals that received (±)-SB 209670 alone during the second clearance period ($n = 5$), animals that received endothelin alone during the second clearance period ($n = 7$), and animals that received both (±)-SB 209670 and endothelin-1 ($n = 7$).

In order to evaluate the effect of (±)-SB 209670 on cyclosporine A-induced renal vasoconstriction, rats were prepared as described above and two 30 min renal clearances performed. At the end of clearance 1, (±)-SB 209670 (30 $\mu$g/kg · min, i.v.; $n = 7$) or its vehicle ($n = 7$) was infused. 5 min later, cyclosporine A (Sandoz) was administered i.v. at a dose of 50 mg/kg. The dose of (±)-SB 209670 used in the present study (30 $\mu$g/kg · min) is a dose that results in an approximate 10-fold shift in the pressor dose-response curve for endothelin-1 (Gellai, unpublished observations) and the dose required to reverse ischemia-induced acute renal failure in the rat (Gellai et al., 1995).

### 2.3. Materials

[$^{14}$C]Inulin and [$^{3}$H]*p*-aminohippuric acid were obtained from Amersham. Cyclosporine A (Sandimmune) was made by Sandoz.

### 2.4. Data analysis

Renal plasma flow and glomerular filtration rate were determined as the clearances of *p*aminohippuric acid and inulin, respectively. Data are expressed as means ± S.E.M. and were analyzed statistically using paired and unpaired *t*-tests as appropriate.

## 3. Results

Infusion of endothelin to anesthetized rats resulted in a significant reduction in both renal blood flow and glomerular filtration rate (Fig. 1). Concomitant infusion of the endothelin receptor antagonist, (±)-SB 209670, abolished the endothelin-induced renal vasoconstriction (Fig. 1). The dose of endothelin used in the present study had no effect on mean arterial blood pressure, however, it resulted in a significant increase in renal vascular resistance which was abolished by (±)-SB 209670 (Fig. 2). Neither heart rate nor urine flow were altered significantly by endothelin infusion

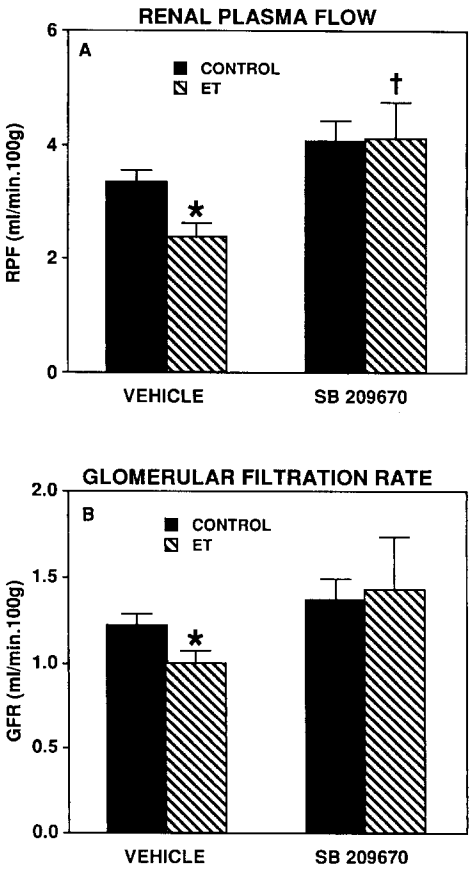
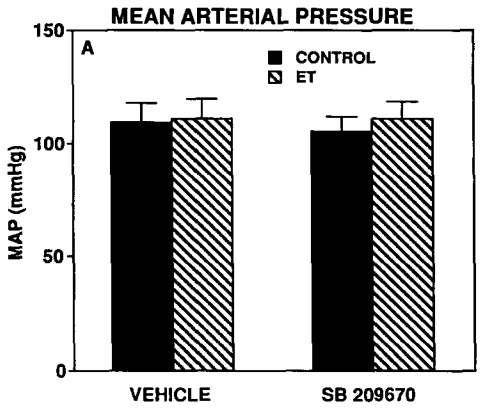



Fig. 1. Renal plasma flow (RPF; A) and glomerular filtration rate (GFR; B) of inactin-anesthetized rats administered with endothelin-1 (ET; 100 pmol/kg, i.v.) in the presence or absence of the endothelin receptor antagonist, (±)-SB 209670 (30 $\mu$g/kg · min, i.v.). $n = 7$ rats/group. $^{*}P < 0.05$ vs. control; $^{\dagger}P < 0.05$ vs. vehicle.



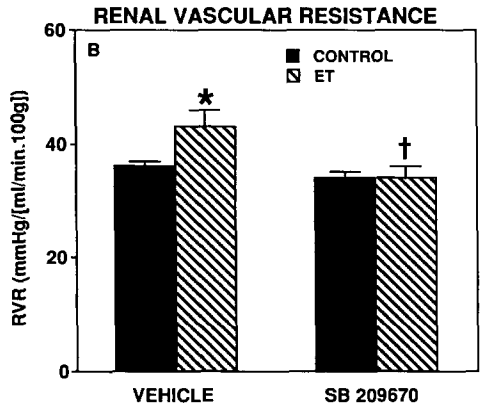
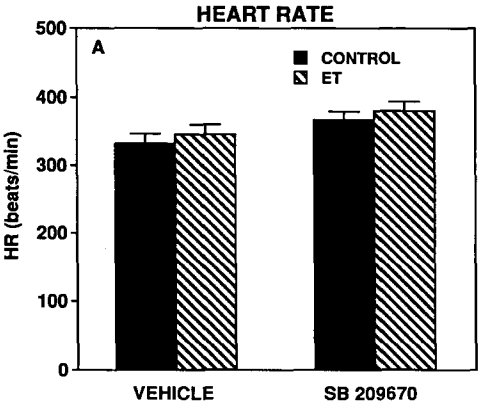




Fig. 2. Mean arterial pressure (MAP; A) and renal vascular resistance (RVR; B) of inactin-anesthetized rats administered with endothelin-1 (ET; 100 pmol/kg, i.v.) in the presence and absence of the endothelin receptor antagonist, (±)-SB 209670 (30 μg/kg·min, i.v.). $n = 7$ rats/group. $^{*}P < 0.05$ vs. control; $^{\dagger}P < 0.05$ vs. vehicle.



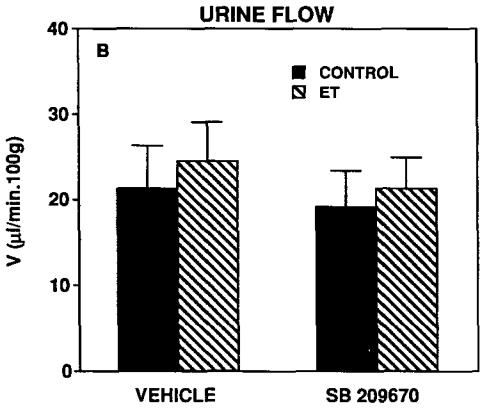




Fig. 3. Heart rate (HR; A) and urine flow (V; B) of inactin-anesthetized rats administered endothelin-1 (ET; 100 pmol/kg, i.v.) in the presence and absence of the endothelin receptor antagonist, (±)-SB 209670 (30 μg/kg·min, i.v.). $n = 6$ rats/group.

(Fig. 3). In time control studies, infusion of (±)-SB 209670 or vehicle alone had no significant effect on any of the parameters measured (Table 1).

Administration of cyclosporine A intravenously resulted in an acute renal vasoconstriction as evidenced by significant reductions in both renal blood flow and glomerular filtration rate (Fig. 4). Since mean arterial pressure did not change, there was an increase in renal vascular resistance (Fig. 5). Infusion of (±)-SB 209670 significantly attenuated cyclosporine A-induced

Table 1
Systemic and renal hemodynamics in inactin-anesthetized rats treated with vehicle (group 1) or the endothelin $ET_A/ET_B$ receptor antagonist, (±)-SB 209670 (group 2)

| | Group 1 | | Group 2 | |
|---|---|---|---|---|
| | Vehicle | Vehicle | Vehicle | (±)-SB 209670 |
| Renal plasma flow (ml/min · 100 g) | 2.9 ± 0.3 | 2.7 ± 0.1 | 3.4 ± 0.7 | 3.4 ± 0.8 |
| Glomerular filtration rate (ml/min · 100 g) | 0.9 ± 0.1 | 0.9 ± 0.1 | 1.1 ± 0.2 | 1.2 ± 0.2 |
| Mean arterial pressure (mm Hg) | 109 ± 6 | 111 ± 6 | 106 ± 9 | 96 ± 7 |
| Renal vascular resistance (mm Hg/[ml/min · 100 g]) | 33 ± 2 | 34 ± 2 | 32 ± 3 | 36 ± 3 |
| Heart rate (beats/min) | 342 ± 9 | 362 ± 9 | 372 ± 20 | 400 ± 14 |
| Urine flow (μl/min · 100 g) | 9.8 ± 2.7 | 17.0 ± 4.7 | 10.3 ± 1.2 | 6.7 ± 1.7 |

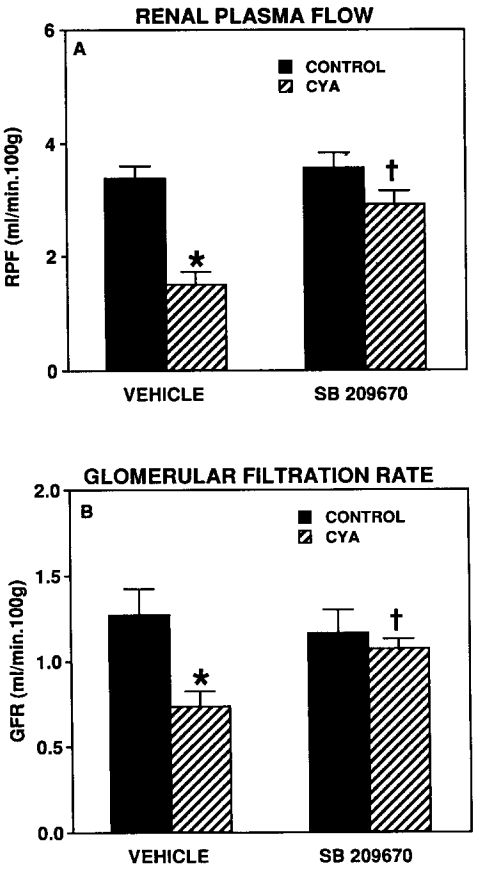



Fig. 4. Renal plasma flow (RPF; A) and glomerular filtration rate (GFR; B) of inactin-anesthetized rats administered cyclosporine A (CYA; 50 mg/kg, i.v.) in the presence or absence of the endothelin receptor antagonist, (±)-SB 209670 (30 μg/kg·min, i.v.). $n = 6$ rats/group. $^{*}P < 0.05$ vs. control; $^{\dagger}P < 0.05$ vs. vehicle.

changes in renal blood flow, glomerular filtration rate, and renal vascular resistance (Figs. 4 and 5). Heart rate was not altered by cyclosporine A (Fig. 6). Cyclosporine A induced a significant reduction in urine flow which was attenuated by (±)-SB 209670 (Fig. 6).

## 4. Discussion

In the present study, we have demonstrated that the novel endothelin receptor antagonist, (±)-SB 209670, at a dose that prevented the renal vasoconstrictor effects of exogenous endothelin-1, attenuated the renal vasoconstriction and fluid retention induced by acute cyclosporine A administration. (±)-SB 209670 is a potent antagonist of both endothelin $ET_A$ and $ET_B$ receptors. (±)-SB 209670 can inhibit [$^{125}$]endothelin-1 binding to human endothelin $ET_A$ and $ET_B$ receptors cloned and stably expressed in Chinese hamster ovary cells with $K_i$ values of 0.2 and 18 nM, respectively (Nambi et al., 1994). (±)-SB 209670 produces parallel rightward shifts in the concentration-response curve for endothelin-1 in the rat isolated aorta which contains endothelin $ET_A$ receptors with a $K_B$ value of 0.41 ± 0.04 nM (Ohlstein et al., 1994). At higher concentrations, (±)-SB 209670 can antagonize endothelin $ET_B$ receptors competitively by producing parallel rightward shifts of the concentration-response curves for endothelin-1 in the rabbit isolated pulmonary artery with a $K_B$ value of 199 ± 9 nM (Ohlstein et al., 1994). (±)-SB 209670 has been shown to be effective in preventing or reversing ischemia-induced acute renal failure in the rat (Gellai et al., 1995) and dog (Brooks et al., 1994a) and in radiocontrast nephrotoxicity in the dog (Brooks et al., 1995).

The present study indicates that the endothelin receptor antagonist, (±)-SB 209670, may also be effective in cyclosporine A nephrotoxicity. The ability of (±)-SB 209670 to inhibit the acute renal vasoconstrictor effects of cyclosporine A is consistent with previous findings, demonstrating that blockade of endothelin action with either an endothelin antibody (Kon et al., 1990; Perico et al., 1990; Bloom et al., 1993) or a peptide endothelin receptor antagonist (Fogo et al.,

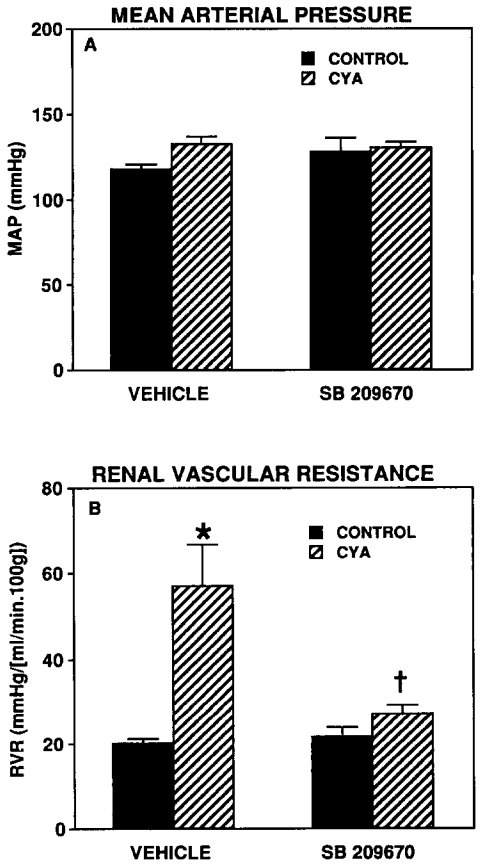



Fig. 5. Mean arterial pressure (MAP; A) and renal vascular resistance (RVR; B) of inactin-anesthetized rats administered cyclosporine A (CYA; 50 mg/kg, i.v.) in the presence or absence of the endothelin receptor antagonist, (±)-SB 209670 (30 μg/kg·min, i.v.). $n = 6$ rats/group. $^{*}P < 0.05$ vs. control; $^{\dagger}P < 0.05$ vs. vehicle.

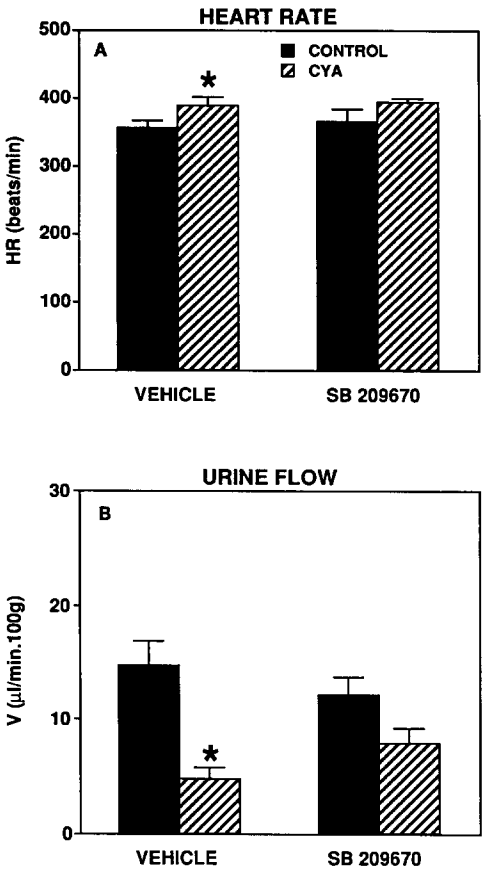



Fig. 6. Heart rate (HR; A) and urine flow (V; B) of inactin-anesthetized rats administered cyclosporine A (CYA; 50 mg/kg, i.v.) in the presence or absence of the endothelin receptor antagonist, (±)-SB 209670 (30 µg/kg·min, i.v.). $n = 6$ rats/group. $^*P < 0.05$ vs. control.

1992; Conger et al., 1994; Lanese and Conger, 1993) can block cyclosporine A-induced vasoconstriction in vivo and in isolated renal arterioles. In the studies conducted in isolated renal arterioles, Lanese and Conger (1993) were able to demonstrate the afferent arteriole was more sensitive to the vasoconstrictor effects of cyclosporine A than the efferent arteriole. Furthermore, the vasoconstrictor effects of cyclosporine A were mediated by endothelin in the afferent arteriole but not the efferent arteriole.

Previous studies using peptide receptor antagonists involved the selective endothelin $ET_A$ receptor antagonist, BQ123, suggesting that the endothelin $ET_A$ receptor antagonistic property of (±)-SB 209670 may be the mechanism by which (±)-SB 209670 prevents the renal vasoconstrictor effects of cyclosporine A. To our knowledge, this is the first study demonstrating that a nonpeptide endothelin $ET_A/ET_B$ receptor can inhibit the vasoconstrictor effects of cyclosporine in vivo. This is an important observation since stimulation of endothelin $ET_B$ receptors in vivo can result in nitric oxide-induced vasodilation and thus blockade of endothelin $ET_B$ receptors could enhance the vasoconstrictor effects of endothelin. Since (±)-SB 209670 was effective in reducing cyclosporine A-induced vasoconstriction in vivo, it is apparent that inhibition of endothelin $ET_B$ receptor-induced vasodilation is not significant. It is also possible that endothelin $ET_B$ receptor-mediated vasoconstriction may play a role in cyclosporine-induced vasoconstriction since there is growing evidence that endothelin-induced renal vasoconstriction in the rat may be mediated by endothelin $ET_B$ receptors (Cristol et al., 1993; Pollock and Opgenorth, 1993), in contrast to the dog where no endothelin $ET_B$ receptor-mediated vasoconstriction can be observed (Brooks et al., 1994b). Furthermore, the renal endothelin binding observed in cyclosporine A-treated rats (Nambi et al., 1990; Awazu et al., 1991) appears to involve a preferential increase in the endothelin $ET_B$ receptor since endothelin $ET_B$ receptor mRNA but not endothelin $ET_A$ receptor mRNA is increased following cyclosporine A treatment (Takeda et al., 1994). It has been suggested that there may be two subtypes of the endothelin $ET_B$ receptor, a putative endothelin $ET_{B1}$ subtype which is similar to the human cloned endothelin $ET_B$ receptor and mediates endothelin-induced vasodilation, and a putative endothelin $ET_{B2}$ receptor that mediates vasoconstriction (Warner et al., 1993). Whether $ET_B$-mediated vasoconstriction contributes to cyclosporine A-induced vasoconstriction in the rat is not clear and will only be clearly demonstrated when selective inhibitors of endothelin $ET_{B2}$ receptor-mediated vasoconstriction have been identified.

In the present study, endothelin alone did not alter urine flow, despite a significant decrease in renal blood flow and glomerular filtration rate. It is possible that direct tubular effects of endothelin to reduce sodium and water reabsorption offset the effects of reduced glomerular filtration rate. Cyclosporine A caused a significant reduction in urine flow which was attenuated in part by (±)-SB 209670. The effect of (±)-SB 209670 on cyclosporine-induced fluid retention was modest and may have reflected the improved glomerular filtration rate.

In summary, therefore, our data indicate the novel nonpeptide endothelin $ET_A$/endothelin $ET_B$ receptor antagonist, (±)-SB 209670, can prevent the acute renal effects of cyclosporine A.

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