Effects of the antihypertensive agent, cicletanine, on noradrenaline release and vasoconstriction in perfused mesenteric artery of SHR

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1   The mechanism by which cicletanine (CIC) exerts its antihypertensive effects has not been fully elucidated. The present study was undertaken to examine the effects of *in vivo* and *in vitro* treatment with CIC on the pressor response and noradrenaline (NA) overflow during periarterial nerve stimulation (PNS) in perfused mesenteric arterial beds isolated from spontaneously hypertensive rats (SHR).

2   CIC at a dose of 50 mg kg^{-1} day^{-1} was administered orally to both SHR and normotensive Wistar-Kyoto rats (WKY) from the 6th to 10th week of age. At the 10th week, the isolated mesenteric arterial bed was perfused with Krebs-Henseleit buffer and changes in perfusion pressure and NA overflow during PNS were measured.

3   Chronic treatment with CIC suppressed the age-related elevation of systemic blood pressure in SHR but not in WKY.

4   The PNS (20 Hz)-induced mesenteric vasoconstrictor response and NA overflow were greater in SHR than in WKY. In the vasculature of SHR chronic treatment with CIC resulted in a significant attenuation of the vasoconstriction and the NA overflow during PNS, whereas it did not alter vasoconstrictor responses to bolus injections of KCl and phenylephrine.

5   Treatment with 30 μM CIC *in vitro* diminished the PNS-induced vasoconstriction and NA overflow but not the NA- and KCl-induced vasoconstriction in the vasculature of untreated SHR.

6   In the vasculature of SHR PNS-induced NA overflow was attenuated by prostaglandin E$_2$ (0.05 μM), whereas it was augmented by the cyclo-oxygenase inhibitor diclofenac-Na (30 μM). In the presence of diclofenac, *in vitro* treatment with CIC did not attenuate the NA overflow during PNS.

7   The results suggest that the antihypertensive effect of CIC in SHR is partially due to the presynaptic inhibition of NA release during sympathetic nerve activation. Transjunctional inhibition of NA release by prostaglandins may contribute to the inhibitory action of CIC on NA release in the vasculature of SHR.

Keywords: Cicletanine; perfused mesenteric artery; periarterial nerve stimulation; noradrenaline overflow; prostaglandin; spontaneously hypertensive rats

Introduction

The antihypertensive agent cicletanine (CIC), a furopyridine derivative, exhibits several pharmacological actions on vascular vessels including activation of prostacyclin synthesis (Dorian *et al.*, 1984; 1988; Calder *et al.*, 1992a,b), histamine H$_1$-receptor blockade (Schoeffter *et al.*, 1987; Schoeffter & Godfraind, 1988), α_1-adrenoceptor blockade (Chabrier *et al.*, 1988), calcium channel blockade (Noack & Deitmer, 1993), opening of potassium channels (Koltai *et al.*, 1990), inhibition of guanosine 3′:5′-cyclic monophosphate (cyclic GMP) phosphodiesterase (Silver *et al.*, 1990; 1991), and direct relaxation of vascular smooth muscle (Auguet *et al.*, 1988). The mechanism underlying the antihypertensive action of CIC is complex and not fully understood.

Long-term treatment with CIC reduces systemic blood pressure in spontaneously hypertensive rats (SHR) (Auguet *et al.*, 1988; Jin *et al.*, 1991; Ando *et al.*, 1994) and deoxycorticosterone acetate (DOCA)-salt hypertensive rats (Fuentes *et al.*, 1989; Castro *et al.*, 1990) in which sympathetic nerve activity is increased (Judy *et al.*, 1976; Fujita *et al.*, 1983). Acute treatment with CIC also reduced blood pressure in rats with stress induced by social deprivation (Castro *et al.*, 1988).

Sympathetic nerve activity of these rats is also considered to be elevated. In addition, Ando *et al.* (1994) have shown that CIC reduced blood pressure of SHR fed with a high salt diet, which exhibited increased sympathetic nerve activity. The reduction was accompanied by a decrease in the plasma concentration of catecholamines (Ando *et al.* 1994). These findings suggest that CIC exerts its antihypertensive effect through suppression of an elevated sympathetic tone. However, it is not known whether CIC acts directly on sympathetic nerve terminals, resulting in inhibition of noradrenaline (NA) release upon nerve stimulation.

The present study was undertaken to elucidate the possible mechanism of the inhibition of sympathetic tone by CIC. For this purpose, the *ex vivo* and *in vitro* effects of CIC on vasoconstriction and NA overflow during periarterial nerve stimulation (PNS) in the mesenteric arterial bed isolated from SHR were examined. In this preparation, electrical nerve stimulation results in an overflow of NA that is greater in SHR than in Wistar-Kyoto rats (WKY) (Ekas & Lokhandwala, 1981; Tsuda *et al.*, 1984). Furthermore, vascular reactivity to exogenous NA is enhanced in the mesenteric arterial bed in SHR (Ekas *et al.*, 1983). Thus, the preparation is appropriate for studying the effect of CIC on the sympathetic nerve system. If an inhibitory action of CIC on sympathetic transmitter

release contributes to its antihypertensive action, both vasoconstriction and NA overflow during PNS should be reduced in vessels from animals treated with CIC. In addition, since the α_1-adrenoceptor blocking action of CIC has been shown to be weak (Auguet *et al.*, 1988), the vasoconstriction to PNS should be reduced by CIC more effectively than that to exogenous vasoconstrictor agents. It has been shown that CIC increases the plasma concentrations of prostaglandin E_2 (PGE_2) and prostaglandin I_2 (Yasu *et al.*, 1995), and PGE_2 is known to inhibit NA release via the activation of presynaptic PGE_2 receptors (Tsuda *et al.*, 1987; Rump *et al.*, 1990). For this reason, the possible involvement of prostaglandin-mediated inhibition of NA release in the action of CIC was also examined.

Methods

Animals

Six week-old male SHR and normotensive WKY (Charles River Japan, Atsugi, Japan) were used in the present study. The rats were fed with a regular diet, allowed free access to tap water and acclimatized in a quarantine room for at least 1 week before experiments. The animal protocol was designed according to the Guideline of Experimental Animal Care issued from the Prime Minister's Office of Japan and approved by the Animal Care and Use Committee of the University.

The SHR were randomly divided into three groups: (1) SHR treated with CIC for 4 weeks to assess the chronic effect, (2) SHR treated with the CIC vehicle for 4 weeks, and (3) age-matched untreated SHR to examine the direct effect of CIC on the mesenteric arterial bed and the possible involvement of prostaglandins in modulating NA overflow. The WKY were also divided into three groups, CIC-treated WKY, vehicle-treated WKY, and age-matched untreated WKY.

Measurement of blood pressure

Systolic blood pressure and heart rate of conscious rats were determined by the tail-cuff method by use of a blood pressure analyser (BP-98A, Softron, Tokyo, Japan) connected to a personal computer (PC9801 series, NEC, Japan). An average of three successive recordings was made once a week, 3–4 h after the oral administration of the agent. In a preliminary study, we found that the antihypertensive effect of CIC was maximal 3–4 h after administration.

Perfused mesenteric arterial preparation

The perfused mesenteric arterial bed was prepared according to McGregor (1965). The rats were anaesthetized with nitrous oxide, oxygen (3:1) and 2.0% halothane. After laparotomy, the superior mesenteric artery was dissected and cleaned of surrounding tissue. A stainless-steel cannula (21G syringe, external diameter 0.80 mm, Terumo, Tokyo, Japan) was inserted into superior mesenteric artery, and the preparations were perfused with Krebs-Henseleit solution described below. After cannulation, branches of mesenteric arteries to the descending colon proximal to the rectum, those to the duodenum proximal to the stomach, those to the caecum and the inferior mesenteric artery branches were tied off. Then, the entire mesenteric vasculature was separated from the small intestine. The preparation was placed in a chamber with a water jacket maintained at 37°C and covered with cotton gauze to keep it moist. The preparation was perfused with Krebs-Henseleit solution equilibrated with a mixture of 95% O_2 and 5% CO_2 ($PO_2 > 600$ mmHg). The Krebs-Henseleit solution consisted of (mM): NaCl 118, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_2 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25, EDTA-Na_1 0.025 and glucose 11 (pH 7.4). The perfusion flow rate was maintained at 2.0 ml min^{-1} with a peristaltic pump (Perista Pump, SJ-1211, Atto, Co., Ltd., Tokyo, Japan). Perfusion pressure was measured through a branch of the perfusion cannula by means of a pressure transducer (TP-400T, Nihon Kohden, Tokyo, Japan) connected to a carrier amplifier (AP-621G, Nihon Kohden, Tokyo, Japan) and recorded on a thermal pen-recorder (WT-645G, Nihon Kohden, Tokyo, Japan). The preparation was equilibrated for 30 min before the onset of each experiment.

Periarterial nerve stimulation (PNS)

Two platinum electrodes were used to apply periarterial nerve stimulation (PNS); one was placed at the cannula inserted into the superior mesenteric artery and the other at the peripheral part of the mesenteric arterial bed. PNS was applied with an electrical stimulator (SEN-330, Nihon Kohden, Tokyo, Japan) connected to an electrical isolator (SS-302J, Nihon Kohden, Tokyo, Japan). For pressor responses to PNS, rectangular pulses (amplitude of 6 V and pulse duration of 3 ms) were applied at a frequency of either 5, 10, 20 or 40 Hz. The preparation was stimulated for 30 s at 5 min intervals. To determine the overflow of NA during PNS the mesenteric arterial bed was stimulated at 20 Hz for 30 s (rectangular pulses of 3 ms duration) and the perfusate was collected.

In a preliminary study, we observed that frequency-dependent vasoconstriction in the vasculature of SHR was substantially blocked by 0.3 μM tetrodotoxin and 10 μM guanethidine (data not shown). This is in agreement with previous observations by others (Kawasaki & Takasaki, 1984; Tsuda *et al.*, 1984; Li & Duckles, 1992). The concentrations of tetrodotoxin and guanethidine employed here did not block the pressor response to exogenous NA. Although in the rat mesentric vasculature ATP may contribute to the pressor response, we determined only NA overflow during PNS.

Chronic treatment with cicletanine

Cicletanine (CIC, 50 mg kg^{-1}), suspended in 0.25% sodium carboxymethyl cellulose (CMC-Na) solution with the aid of a sonicator, was administered orally through a sonde (0.2 ml 100 g^{-1} rat body weight) at noon of each day from the 6th to 10th week of age. Systolic blood pressure was measured once a week, 3–4 h after administration of CIC. As vehicle-treated groups, vehicle suspended with 0.25% CMC-Na (0.2 ml 100 g^{-1} rat body weight) was administered. Four weeks later, the perfused mesenteric vascular bed was prepared. In these preparations, pressor response and NA overflow caused by PNS at 20 Hz were measured, and then pressor responses to KCl and phenylephrine were determined.

In vitro exposure to cicletanine

Ten week-old, untreated SHR and WKY were used. To assess the *in vitro* effects of CIC, the mesenteric arterial bed was perfused with buffer containing either 30 or 100 μM CIC or vehicle (0.02% dimethylsulphoxide, DMSO). According to the results of Bukoski *et al.* (1993), CIC at the concentration of 100 μM or more elicits significant vasorelaxation of mesenteric resistance arteries of SHR precontracted with NA. In contrast, 0.1 to 10 μM of CIC did not significantly affect the vascular

response to NA and other vasoconstrictors (Silver *et al.*, 1991; Deitmer *et al.*, 1992; Bukoski *et al.*, 1993). Thus, we employed two concentrations of CIC, 30 and 100 μM, in the *in vitro* study. In the first series of experiments, pressor response and NA overflow caused by PNS at 20 Hz were measured in the presence of 30 μM CIC, and then pressor responses to KCl and NA were determined. In the second series of experiments, a frequency-response curve to PNS and dose-responses curve to exogenous KCl and NA were determined in the presence of 100 μM CIC. CIC was perfused from 30 min before the first application of PNS to the end of the experiment. Potassium chloride, phenylephrine and NA solutions were injected in the perfusion buffer at a volume of 50 μl. The bolus injection of 50 μl of vehicle into the perfusing solution produced little effect on perfusion pressure.

Role of prostaglandins

To examine the possible involvement of prostaglandins in the response to CIC, the effects of PGE_2 and diclofenac sodium (DCF), a cyclo-oxygenase inhibitor, were studied in mesenteric arterial beds isolated from 10 week-old SHR. In a preliminary study, attenuation of NA overflow by PGE_2 and enhancement by DCF were not observed when no NA transport inhibitor was in the perfusate. This observation is in agreement with a previous one (Tsuda *et al.*, 1987). Thus, this series of experiments was performed in the presence of 0.02 μM nisoxetine, a NA transport inhibitor (Richelson & Nelson, 1984; Tejani-Butt, 1992). In these studies the mesenteric preparations were stimulated once at 20 Hz (S1) to stabilize further response to PNS. After a 30 min equilibration period, PNS-induced NA overflow was determined (S2) in the absence of PGE_2 or DCF. The preparations were then treated with 0.05 μM PGE_2 or 30 μM DCF for 1 h, and NA overflow during PNS was determined (S3) in the presence of PGE_2 or DCF. % changes in NA overflow ((S3 – S2)/S2 $\times$ 100) were calculated. As a time-matched control, NA overflow in the absence of PGE_2 or DCF was also determined.

In another set of experiments, the effect of CIC on PNS-induced NA overflow was studied in vascular beds from SHR in the presence or absence of 30 μM DCF. The perfusion protocol was the same as in the PGE_2 and DCF experiments. Thus, the effect of CIC on PNS-induced NA overflow was studied in one preparation. NA overflow was determined (S2) before CIC treatment, and then CIC at 30 μM was applied to the preparation for 1 h followed by determination of NA overflow in the presence of CIC (S3). Similarly, the effect of CIC on NA overflow was studied in the presence of DCF, which was applied during the whole period of the experiment. Both nisoxetine and DCF were present in the Krebs-Henseleit buffer. % changes in NA overflow ((S3 – S2)/S2 $\times$ 100) were calculated. As a time-matched control, NA overflow in the absence of CIC was also determined.

Determination of NA

The perfusate through the mesenteric vasculature was collected in a tube containing 0.1 mg ml^{-1} glutathione as an antioxidant. NA was absorbed on activated alumina at pH 8.3 – 8.5 which was obtained by addition of 2 M ammonium acetate. The alumina was washed twice with distilled water. NA was then extracted from the alumina with 200 μl of 0.2 M perchloric acid containing 0.01% EDTA-Na$_2$. An aliquot was applied to a high-performance liquid chromatography (h.p.l.c.) system with an electrochemical detector (ECD-100, Eicom, Kyoto, Japan) (Hjemdakl *et al.*,

1979). 3,4-Dihydroxybenzylamine was used as an internal standard. NA levels were normalized to g wet tissue weight for each preparation. NA overflow induced by PNS was defined as the difference between the basal NA efflux detected for 3 min before stimulation and the NA level detected during the 30 s of PNS and the subsequent 2.5 min period.

Drugs

The following drugs were used: (−)-phenylephrine hydrochloride, guanethidine monosulphate, 3,4-dihydroxybenzylamine hydrobromide, prostaglandin E$_2$, diclofenac-Na (Sigma, MO, U.S.A.), nisoxetine hydrochloride (Funakoshi Yakuhin, Tokyo, Japan), alumina activated 200 for determination of catecholamine (Nacalai Tesque, Kyoto, Japan), dimethyl-sulphoxide (Kanto Chemical, Tokyo, Japan), (−)-noradrenaline bitartrate, tetrodotoxin (Wako Pure Chemical Industries, Osaka, Japan). Cicletanine hydrochloride was a generous gift from Nippon Roussel (Tokyo, Japan). All agents except CIC were dissolved in distilled water just before use. For the *in vitro* experiment, CIC was dissolved in 0.02% DMSO.

Data analysis

Results are expressed as the means $\pm$ s.e.mean. Two-way analysis of variance (ANOVA) for repeated measures was applied to determine whether systolic blood pressure and heart rate were affected by age and/or CIC treatment. Two-way ANOVA (repeated measures) was also applied to determine whether the pressor response to PNS or vasoconstrictors was affected by frequency or dose and/or CIC treatment. In some cases two-way factorial ANOVA was used to determine whether each parameter was affected by the strain (SHR vs WKY) and/or CIC treatment. *Post-hoc* comparisons were performed by Scheffe's test. Where appropriate, unpaired two-tailed Student's *t* test and paired *t* test were used. In all cases differences with a probability of 5% or less ($P < 0.05$) were considered to be statistically significant. Statistical analysis was performed with StatView software (Abacus Concepts, Berkeley, CA, U.S.A.).

Results

Chronic treatment with CIC

Table 1 shows changes in systolic blood pressure and heart rate in SHR and WKY treated with CIC or vehicle from the 6th to 10th week of age. Chronic treatment with CIC significantly attenuated the rise in systolic blood pressure of the SHR. CIC did not affect systolic blood pressure in WKY. Heart rate in both groups was not affected by CIC.

The effects of chronic treatment with CIC on PNS-induced vasoconstriction and NA overflow are shown in Figure 1. The vasoconstrictor response to PNS at 20 Hz was significantly greater in preparations of SHR than in preparations of WKY ($P = 0.0004$, two-way ANOVA). The greater response was associated with a significantly greater NA overflow in SHR ($P = 0.001$, two-way ANOVA). In the SHR, chronic treatment with CIC resulted in a significant attenuation in both vasoconstriction and NA overflow. In the WKY, treatment with CIC did not alter the PNS-induced vasoconstriction and NA overflow.

The effects of chronic treatment with CIC on KCl- and phenylephrine-induced vasoconstriction are shown in Figure 2. A bolus injection of KCl or phenylephrine resulted in a dose-

Table 1 Changes in systolic blood pressure (SBP) and heart rate (HR) in spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY) treated with cicletanine (CIC, 50 mg kg^{-1} day^{-1}) or vehicle from the 6th to 10th week of age

			SBP (mmHg)				*HR* (beats min^{-1})		
Group	n	6th week	10th week	P *(ANOVA)*		6th week	10th week	P *(ANOVA)*	
SHR vehicle	7	137 ± 3	198 ± 3	Age,	<0.0001	442 ± 7	430 ± 14	Age,	0.342
CIC	7	134 ± 4	182 ± 3	Treat,	0.014	409 ± 20	454 ± 11	Treat,	0.650
P (vehicle vs CIC)		0.954	0.011			0.345	0.425		
WKY vehicle	6	117 ± 5	137 ± 3	Age,	0.001	380 ± 10	338 ± 13	Age,	0.001
CIC	6	115 ± 4	132 ± 4	Treat,	0.422	364 ± 11	307 ± 8	Treat,	0.045
P (vehicle vs CIC)		0.995	0.670			0.869	0.276		

Each value represents the mean $\pm$ s.e.mean. Two-way ANOVA (repeated measures) was used to determine whether SBP or HR was affected by age or treatment (Treat) with vehicle or CIC. Mean values for SBP and HR at each age point were tested for differences between treatments by *post-hoc* comparison with Scheffe's method.
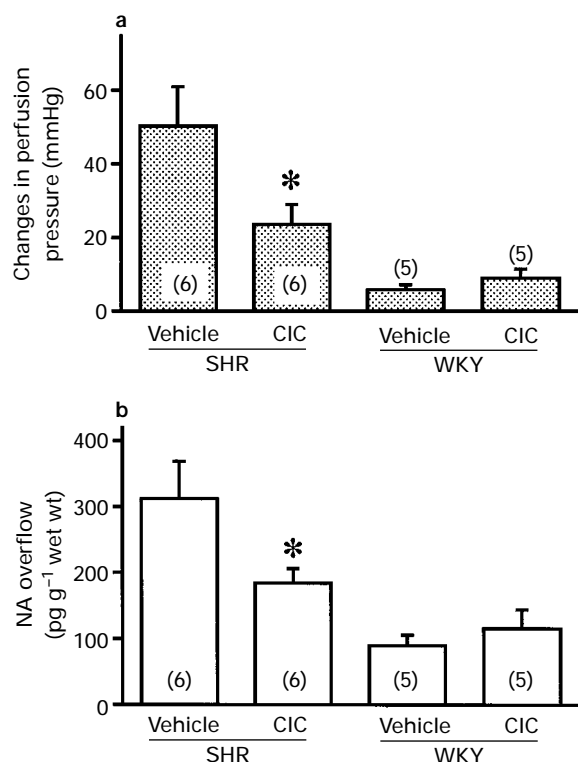

Figure 1 Periarterial nerve stimulation (PNS)-induced pressor response (a) and noradrenaline (NA) overflow (b) in the perfused mesenteric arterial bed of SHR and WKY treated with cicletanine (CIC, 50 mg^{-1} kg^{-1} day) or vehicle for 4 weeks. PNS at 20 Hz was applied to the vascular beds. Data are shown as the means $\pm$ s.e. mean. Numbers in parentheses represent the numbers of animals *P*<0.05 vs corresponding vehicle-treated groups (*post-hoc* comparisons were performed by Scheffe's method).

dependent increase in perfusion pressure. These pressor responses were greater in SHR than in WKY ($P = 0.0001$ and $P = 0.001$, KCl- and phenylephrine-induced responses, respectively, two-way ANOVA). In contrast to the pressor response to PNS, the reactivity of the mesenteric arterial bed of SHR to KCl and phenylephrine was not influenced by chronic treatment with CIC. Similarly, in WKY neither KCl- nor phenylephrine-induced vasoconstriction was reduced after CIC treatment.

In vitro *effects of CIC*

The *in vitro* effects of CIC (30 μM) on vasoconstrictor response to PNS, KCl and exogenous NA are shown in Figure 3.
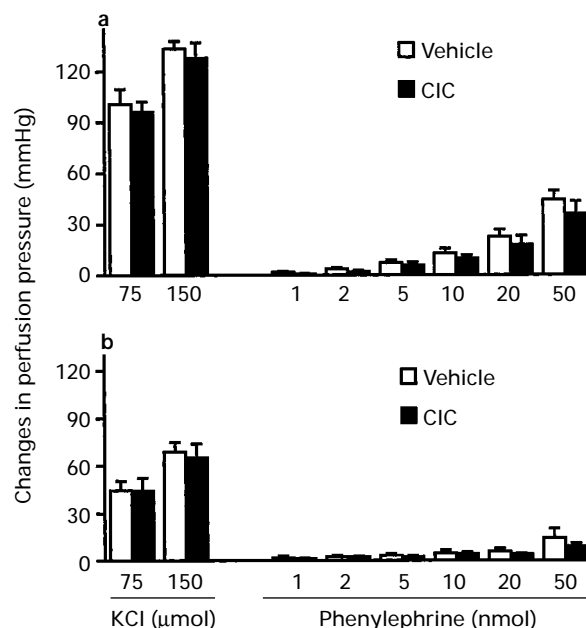

Figure 2 Potassium chloride- and phenylephrine-induced pressor response in the perfused mesenteric arterial bed of SHR (a) and WKY (b) treated with cicletanine (CIC, 50 mg^{-1} kg^{-1} day) or vehicle for 4 weeks. Potassium chloride (KCl) or phenylephrine at doses of $75 - 150$ μmol or $1 - 50$ nmol, respectively, was injected into the perfusate in a volume of 50 μl. Data are shown as the means $\pm$ s.e. mean of $5 - 6$ animals for each group. There were no significant differences between vehicle and CIC-treatment (two-way ANOVA).

Electrical stimulation resulted in a frequency-dependent pressor response. Infusion of CIC (30 μM) *per se* did not affect the basal perfusion pressure. This concentration of CIC suppressed the PNS-induced vasoconstriction but did not affect the KCl- and exogenous NA-induced vasoconstriction in mesenteric arterial beds of SHR and WKY.

The *in vitro* effects of 30 μM CIC on pressor response and NA overflow of the mesenteric artery of untreated SHR and WKY at the stimulation frequency of 20 Hz are shown in Table 2. The basal efflux of NA was not affected by CIC in either group (data not shown). Two-way ANOVA showed that both pressor response and NA overflow were affected by the strain and CIC treatment. In the mesenteric preparation of SHR, PNS-induced NA overflow was significantly reduced by CIC, an effect associated with a marked reduction in the pressor response to PNS. In the vasculature of WKY, the pressor response only tended to be decreased by treatment with CIC. PNS-induced NA overflow was not affected by CIC in WKY.

The *in vitro* effects of a higher concentration (100 μM) of CIC on pressor responses to PNS, KCl and NA in
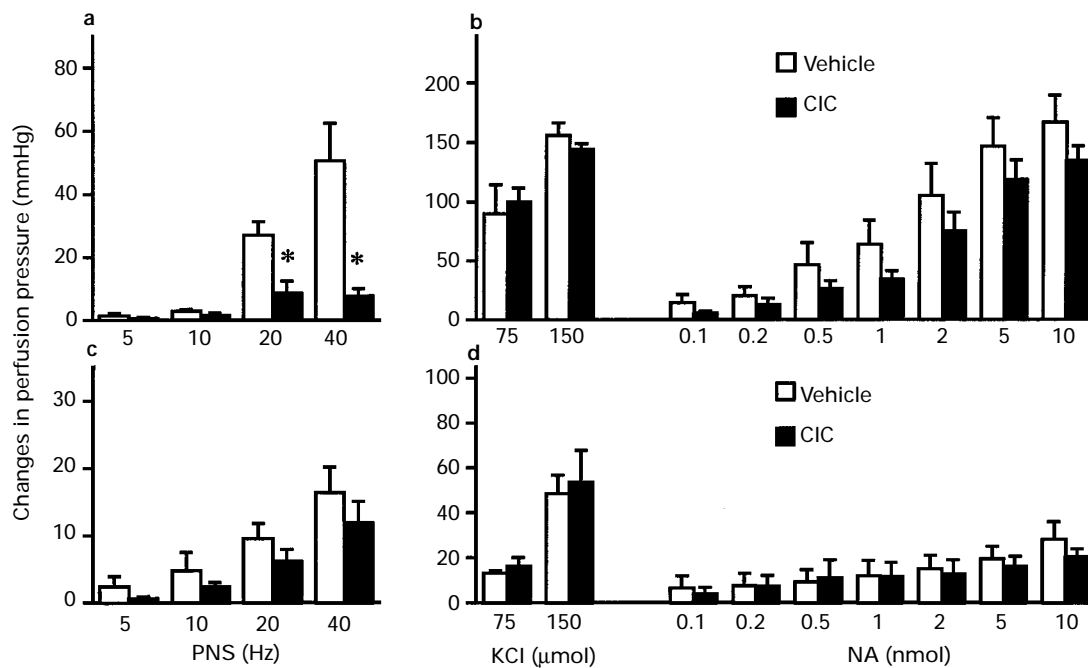
Figure 3 Effects of 30 μM cicletanine (CIC) on pressor responses to PNS (a, c), KCl and noradrenaline (b, d) in the perfused mesenteric arterial beds of (a, b) SHR and (c, d) WKY. The age of the SHR and WKY (10 weeks old) was the same as that of the animals used in Figures 1 and 2. Either CIC or its vehicle was perfused from 30 min before PNS to the end of the experiment. The mesenteric arterial preparation was electrically stimulated at frequencies ranging from 5 to 40 Hz for 30 s. Potassium chloride or noradrenaline at final doses of 75–150 μmol or 0.1–10 nmol, respectively, was injected into the perfusate. Data are shown as the means $\pm$ s.e.mean of 5 animals for each group. *$P < 0.05$ vs corresponding vehicle-treated groups. In (a) and (c), two way factorial ANOVA of the sum of the values of pressor response in both groups showed that PNS-induced pressor response was affected by the strain ($P = 0.033$) and by CIC ($P = 0.001$) whereas two-way ANOVA for repeated measures showed that the PNS-induced pressor response was affected by CIC in SHR ($P < 0.0001$) but not in WKY ($P = 0.283$). In (b), two-way ANOVA for repeated measures showed that pressor response to NA was not affected by 30 μM CIC ($P = 0.279$).

Table 2 *In vitro* effects of 30 μM cicletanine (CIC) on pressor response and noradrenaline overflow during periarterial nerve stimulation (PNS) at 20 Hz in mesenteric arterial beds isolated from untreated SHR and WKY

	Pressor response (mmHg)			*Noradrenaline overflow* (pg g^{-1} wet wt)		
Treatment	*SHR*	*WKY*	P *(ANOVA)*	*SHR*	*WKY*	P *(ANOVA)*
Vehicle	54.6 $\pm$ 12.0 (5)	9.6 $\pm$ 3.1 (7)	Strain, <0.0001 Treat, 0.001	365 $\pm$ 53 (5)	110 $\pm$ 15 (6)	Strain, <0.0001 Treat, 0.001
CIC	19.3 $\pm$ 5.2 (6)	3.0 $\pm$ 0.7 (7)		132 $\pm$ 37 (5)	77 $\pm$ 21 (6)	
P (vehicle vs CIC)	0.005	0.854		0.002	0.894	

Either CIC (30 μM) or its vehicle was infused from 30 min before PNS to the end of the experiments. Each value represents the mean $\pm$ s.e.mean. Numbers in parentheses indicate the numbers of animals. Two-way factorial ANOVA was used to determine whether presssor response or noradrenaline overflow was affected by the strain (Strain) or treatment with vehicle or CIC (Treat). Mean values for pressor response and noradrenaline overflow in each strain, differences between vehicle and CIC treatment were tested by *post-hoc* comparison with Scheffe's method.

mesenteric arterial beds of the untreated SHR are shown in Figure 4. In the presence of 100 μM CIC, the pressor responses to a bolus injection of KCl and NA as well as those to PNS were significantly attenuated in mesenteric arterial beds of the SHR.

Role of prostaglandins

The effects of PGE_2 and DCF on PNS-induced NA overflow in the untreated SHR are shown in Figure 5. PNS-induced NA overflow was significantly attenuated by PGE_2 whereas it was significantly augmented by DCF. The result of time-matched control experiments indicated that the effects of PGE_2 and DCF were not due to time trends. The *in vitro* effects of CIC on

NA overflow in the presence of DCF are shown in Figure 6. The inhibitory effect of 30 μM CIC on NA overflow was not observed in the presence of DCF.

Discussion

Hyperactivity of the sympathetic nervous system in SHR is well-known. In the isolated, perfused mesenteric arterial beds, PNS results in a frequency-dependent increase in perfusion pressure, which is greater in SHR than in WKY (Ekas & Lokhandwala, 1981; Tsuda *et al.*, 1984). PNS-induced vasoconstriction is recognized mainly to be due to endogenous NA released from sympathetic nerve endings

during PNS (Kawasaki & Takanashi, 1984 Tsuda *et al.*, 1984; Li & Duckles, 1992), which activates the postsynaptic α_1-adrenoceptor on the vascular smooth muscle. In addition, the reactivity of vascular smooth muscle to vasoconstrictor agents is also elevated in SHR (Ekas & Lokhandwala,

1981; Tsuda *et al.*, 1984). Thus, the vascular reactivity of SHR to sympathetic nervous stimulation is augmented at both presynaptic and postsynaptic sites and the increased vascular reactivity contributes to the development of hypertension in SHR.
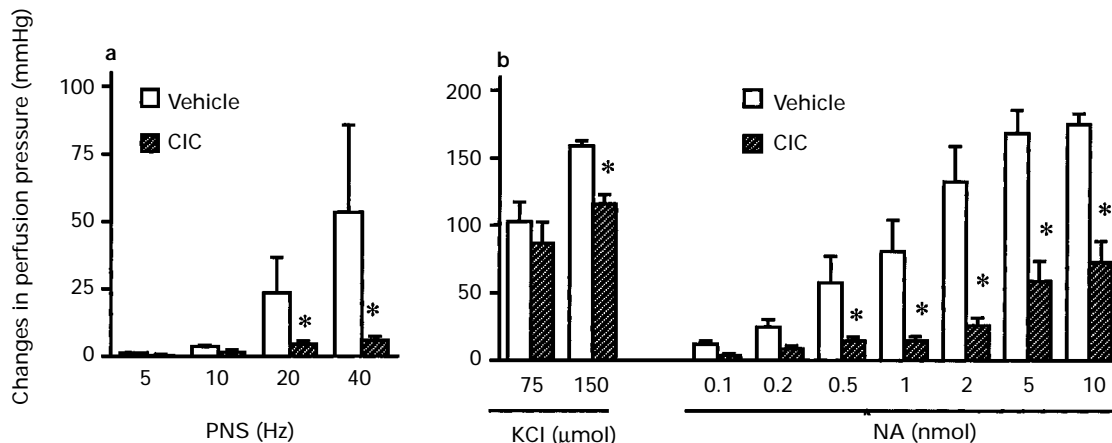
Figure 4 Effects of 100 μM cicletanine (CIC) on pressor responses to PNS (a), KCl and noradrenaline (b) in the perfused mesenteric arterial beds of SHR. The age of the SHR (10 weeks-old) was the same as that of animals used in Figure 3. Either CIC or vehicle was perfused from 30 min before PNS to the end of the experiment. Experimental protocol was the same as that in Figure 3. Data are shown as the means±s.e.mean of 5 animals for each group. *$P < 0.05$ vs corresponding vehicle-treated groups.
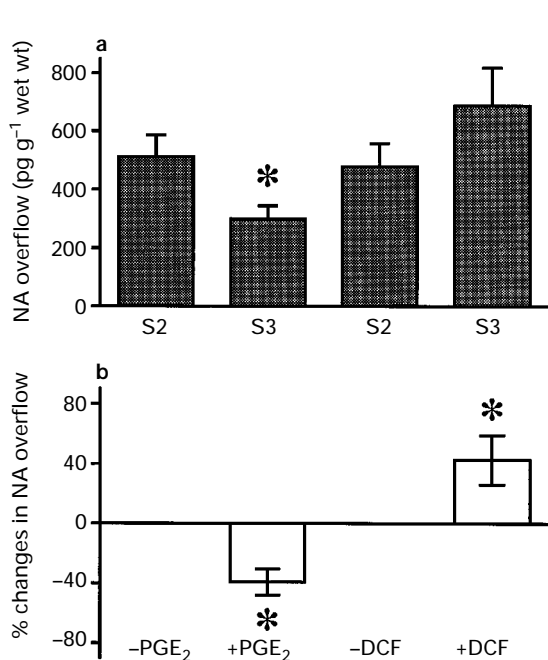
Figure 5 Effects of prostaglandin E_2 (PGE$_2$) and diclofenac sodium (DCF) on noradrenaline (NA) overflow during PNS in the mesenteric arterial bed of SHR. The overflow of NA during PNS in the absence and presence of PGE$_2$ or DCF (S2 and S3, a) and % changes in NA overflow ((S3−S2)/S2 × 100, b) are shown. Each preparation was perfused with Krebs-Henseleit buffer containing 0.02 μM nisoxetine and subjected to PNS at 20 Hz 3 times. At 30 min after the 1st PNS (S1), NA overflow during PNS in the absence of PGE$_2$ or DCF was determined (S2). Then, the preparation was treated with either 0.05 μM PGE$_2$ or 30 μM DCF for 1 h before the 3rd PNS (S3). In time-matched control experiments (no PGE$_2$, no DCF), there was no significant difference between S2 (468±52 pg g^{-1}, n = 5) and S3 (477±60 pg g^{-1}, n = 5). Data are shown as the means±s.e.mean of 6–7 animals for each group. *$P < 0.05$ vs corresponding control.
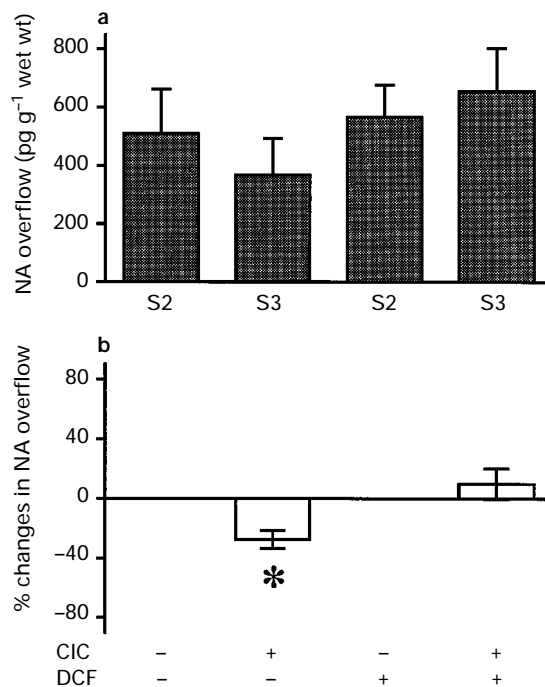
Figure 6 Effects of cicletanine (CIC) on noradrenaline (NA) overflow during PNS in the absence or presence of 30 μM diclofenac sodium (DCF). The overflow of NA during PNS before (S2) and after treatment with 30 μM CIC (S3) in the absence and presence of DCF (a) and % changes in NA overflow ((S3−S2)/S2 × 100, b) are shown. Each preparation was perfused with Krebs-Henseleit buffer containing 0.02 μM nisoxetine and subjected to PNS at 20 Hz 3 times. At 30 min after the 1st PNS (S1), NA overflow during PNS in the absence of CIC was determined (S2). Then, the preparation was treated with CIC for 1 h before the 3rd PNS (S3). In time-matched control experiments (no CIC), there was no significant differences between S2 (484±69 pg g^{-1}, n = 5) and S3 (469±74 pg g^{-1}, n = 5). Data are shown as the means±s.e.mean of 5–7 animals for each group. *$P < 0.05$ vs corresponding control.

To examine the effect of CIC on the sympathetic nervous system, we measured PNS-induced pressor responses of mesenteric arterial beds in SHR and WKY. We also measured NA levels in the effluent during PNS. Our hypothesis is that, if CIC possesses a direct inhibitory action on NA release upon stimulation, both pressor response and NA overflow would be reduced in SHR treated with CIC.

The major finding of the present study was that both pressor response and NA overflow during PNS of the mesenteric arterial beds were attenuated by long-term treatment with CIC in SHR, but not in WKY. This was also associated with suppression of the elevation in systolic blood pressure only in SHR. CIC has been shown to exert an antihypertensive effect in SHR in which sympathetic nerve activity is high (Jin *et al.*, 1991; Ando *et al.*, 1994). Our results suggest that long-term treatment with CIC suppresses the elevated NA release in peripheral vascular beds, and this action of CIC contributes to its antihypertensive effect in SHR.

High doses of KCl act directly on vascular beds and depolarize them followed by vasoconstriction. Phenylephrine is an α_1-adrenoceptor agonist. The present study showed that pressor responses to KCl and phenylephrine in the vasculature of SHR were greater than those of WKY, and these responses were not attenuated by chronic treatment with CIC. Thus, it is suggested that long-term treatment with CIC does not affect the increased reactivity of the vascular smooth muscle in SHR to these vasoconstrictors.

Acute *in vitro* treatment with CIC at a concentration of 30 μM inhibited PNS-induced vasoconstriction more effectively than KCl- or exogenous NA-induced vasoconstriction. This suggests that CIC preferentially suppresses the elevated sympathetic nerve activity in the SHR as compared with its effects on the enhanced reactivity of vascular smooth muscle. The results further suggest that the sympathetic nervous system is a target for the CIC-induced antihypertensive action in SHR. This is in agreement with the hypothesis of other investigators, that CIC exhibits a potent antihypertensive effect in animals with enhanced sympathetic nerve activity such as salt-sensitive SHR (Jin *et al.*, 1991; Ando *et al.*, 1994). Since neither KCl- nor phenylephrine-induced vasoconstriction was attenuated by acute (CIC at 30 μM) or chronic treatment with CIC, the agent appears to have only minor effects on depolarization-induced or α_1-adrenoceptor-mediated pressor responses in the vasculature of SHR.

A higher concentration (100 μM) of CIC reduced vasoconstrictor responses to KCl and NA as well as those to PNS. Auguet *et al.* (1988) showed that 100 μM CIC reduced both NA- and angiotensin II-induced vasoconstriction, suggesting a direct vasodilator action of CIC on vascular smooth muscle. As mentioned in Methods, CIC at the concentration of 100 μM or more exerts a direct vasodilator effect in the mesenteric artery of SHR precontracted with NA (Bukoski *et al.*, 1993). Therefore, high concentrations of CIC may exert direct vasorelaxant effects in SHR vessels, possibly through mechanisms other than an inhibitory action on NA release.

NA release from sympathetic nerve endings is locally regulated by a variety of endogenous substances, such as prostaglandins, angiotensin II, adenosine and released NA itself. These vasoactive substances activate presynaptic receptors. Altered prejunctional control mechanisms may modify NA release in the peripheral vascular beds of SHR and this may contribute to the pathogenesis of hypertension and the antihypertensive mechanism of CIC. It has been shown that plasma levels of PGE_2 and the urinary level of PGE_2 are increased after CIC administration in patients with essential hypertension (Yasu *et al.*, 1995; Guinot & Frolich, 1985). Rump *et al.* (1990) suggested that in rat kidney there is a transjunctional PGE_2 inhibition of NA release, i.e., NA released from nerve endings may induce the local formation and release of PGE_2 which then transjunctionally inhibits further NA release via activation of presynaptic PGE_2 receptors. Thus, it is possible that CIC reduces NA overflow through the increased production of PGE_2. In the present study, we showed that the overflow of NA was reduced by exogenously administered PGE_2 while it was enhanced by the cyclo-oxygenase inhibitor DCF. The latter finding suggests that endogenous prostaglandins, which can modulate NA overflow during PNS, are produced in the isolated mesenteric arterial bed. In fact, it has been observed that considerable amounts of PGE_2 are produced spontaneously within this preparation and that the production is increased by exogenous NA (Pipili & Poyser, 1982). Thus, it is likely that enhanced production and release of prostaglandins such as PGE_2 play a role in the inhibitory effect of CIC on NA overflow. This is supported by the finding that the inhibitory effect of CIC *in vitro* on NA overflow during PNS was abolished by DCF, consistent with the results of Castro *et al.* (1989) that pretreatment with indomethacin blunted the antihypertensive effect of CIC. In addition, CIC has been shown to increase prostacyclin production in vascular smooth muscle cells (Dorian *et al.*, 1984; 1988; Calder *et al.*, 1992a; 1992b). However, the inhibitory effect of prostacyclin on NA release is less potent than that of PGE_2 (Weitzell *et al.*, 1978). Thus, the contribution of prostacyclin to the effect of CIC on NA release may be small.

It must be noted that although exogenously administered PGE_2 diminished NA overflow during PNS in the mesenteric arterial preparation, PGE_2 enhances pressor responses of mesenteric arterial beds to NA and PNS (Jackson & Campbell, 1980). The mechanism of the latter phenomenon remains unclear. Since CIC diminished both vasoconstriction and NA overflow during PNS, the mechanism by which CIC inhibits NA release cannot be explained merely by the enhanced production of PGE_2. Several other mechanisms by which CIC reduces the sympathetic nerve activity have been suggested, including inhibition of cyclic GMP phosphodiesterase (Silver *et al.*, 1990; 1991), opening of potassium channels (Calder *et al.*, 1992a) and calcium channel blockade (Noack & Deitmer, 1993) in sympathetic nerve endings. Silver *et al.* (1991) demonstrated that the potentiating effect of CIC on sodium nitroprusside-induced vasorelaxation was not altered in the presence of a cyclo-oxygenase inhibitor. This cyclo-oxygenase inhibitor-resistant component may also contribute to the antihypertensive mechanisms of CIC.

In conclusion, we demonstrated that both *in vivo* and *in vitro* treatment with CIC reduced NA overflow during sympathetic nerve stimulation. The results suggest that chronic treatment with CIC diminishes the increased sympathetic nerve activity through inhibition of NA release from nerve endings. Enhanced production and release of prostaglandins may contribute to the mechanism by which CIC reduced NA release from sympathetic nerve endings of vascular beds in SHR. This action may play an important role in the antihypertensive effect of CIC in hypertensive animals.

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