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Effects of niflumic acid on α_1-adrenoceptor-induced vasoconstriction in mesenteric artery in vitro and in vivo in two-kidney one-clip hypertensive rats

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Abstract

The influence of niflumic acid (3 and 10 μM), a Cl^- channel antagonist, on cirazoline-induced vasoconstriction in isolated perfused mesenteric artery (5 ml/min) from two-kidney one-clip (2K1C) hypertensive and sham normotensive rats was examined. In addition, the effect of a single i.v. bolus injection of niflumic acid (3 mg/kg) on cirazoline-mediated reduction in vascular conductance in superior mesenteric artery was determined in pentobarbital-anaesthetized hypertensive and normotensive rats. Bolus injections of cirazoline induced a dose-dependent transient increase in the perfusion pressure in vitro. In the presence of niflumic acid, cirazoline-mediated vasoconstriction was significantly inhibited. Cirazoline-induced vasoconstriction in isolated mesenteric beds was also significantly inhibited following perfusion with Cl^--free buffer. Pre-perfusion of mesenteric blood vessels with Cl^--free buffer resulted in a significantly greater inhibition of cirazoline-mediated vasoconstriction in sham normotensive rats than in hypertensive rats. We found that in Cl^--free buffer, cirazoline-mediated vasoconstriction could be further inhibited by niflumic acid. Intravenous infusion of cumulative doses of cirazoline in vivo caused a dose-dependent decrease in superior mesenteric vascular conductance. Pretreatment with niflumic acid significantly impaired cirazoline-mediated decreases in vascular conductance. Our results indicate that chloride ions play an important role in α_1-adrenoceptor-mediated vasoconstriction in mesenteric blood vessels. In addition, the contribution of chloride ions in α_1-adrenoceptor-mediated vasoconstriction in blood vessels from hypertensive rats appears to be reduced.

Keywords: α_1-Adrenoceptor; Chloride ion; Niflumic acid; Two-kidney one-clip hypertensive rat; Mesenteric blood vessel

1. Introduction

In vascular smooth muscle, α_1-adrenoceptor-induced depolarization is believed to occur, in part, as a result of efflux of chloride ions (Walhström, 1973; Van Helden, 1988; Byrne and Large, 1988a). In addition, changes in potassium conductance and the activation of non-specific cation channels also appear to contribute to smooth muscle depolarization after the activation of α_1-adrenoceptors (Byrne and Large, 1988b; Suzuki, 1981). Recently, a report by Criddle et al. (1996) indicated that niflumic acid, a putative Cl^- channel antagonist (Greenwood and Large, 1995), was capable of inhibiting noradrenaline-stimulated contractions in isolated rat aorta. Moreover, it was also suggested that Ca^{2+}-activated Cl^- channels resulted in the opening of voltage-gated nifedipine-sensitive Ca^{2+} channels secondary to stimulation of α-adrenoceptors (Criddle et al., 1996). Therefore, at present, the evidence in the literature indicates that inhibition of Ca^{2+}-activated Cl^- channels affects α-adrenoceptor-mediated functional responses in conduit blood vessels. However, it would appear that in the current literature there is no evidence to indicate whether a Cl^- channel antagonist, such as niflumic acid, is capable of inhibiting α_1-adrenoceptor-mediated vasoconstriction in small resistance blood vessels. Furthermore, there is also no evidence available to indicate whether niflumic acid can affect α-adrenoceptor-mediated vasoconstriction in vivo.

Here, we have (1) examined the influence of niflumic acid on α_1-adrenoceptor-mediated vasoconstriction in isolated, perfused rat mesenteric artery before and after removal of chloride ions, (2) investigated the effects of

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niflumic acid on α_1-adrenoceptor-induced changes in vascular conductance in superior mesenteric artery in pentobarbital-anaesthetized rats, and (3) compared the vascular effects of niflumic acid on α_1-adrenoceptor-stimulated vasoconstriction in two-kidney one-clip (2K1C) hypertensive rats to those of normotensive rats both in vitro and in vivo.

2. Materials and methods

2.1. Surgical preparations

Goldblatt hypertension (2K1C) was induced as described previously (Goldblatt et al., 1934). Briefly, male Sprague-Dawley rats (180–230 g) were anaesthetized with halothane (5% in 100% oxygen for induction; 1% in 100% oxygen for maintenance). After a retroperitoneal flank incision, the left renal artery was dissected free and a U-shape silver clip, with an internal diameter of 0.22 ± 0.01 mm, was placed around the renal artery, close to its junction with the aorta. The wound was closed and bupivacaine (1%) and Cicatrin were applied topically to the site of incision. Sham-operated rats underwent renal artery isolation but no clip was placed on the renal artery. Animals were housed individually with 12 h light/dark cycles and given free access to normal food (Purina rat chow) and tap water. Animals were then randomly selected for experiments.

Four weeks after renal artery clipping or sham operation, animals were anaesthetized with halothane (5% in 100% oxygen for induction; 1% in 100% oxygen for maintenance) and catheters (polyethylene tubing I.D. 0.58 mm, O.D. 0.965 mm) were inserted into the left femoral artery for measurement of arterial blood pressure and removal of blood samples, and the left femoral vein for administration of drugs. The catheters were filled with heparinized saline (25 IU/ml in 0.9% NaCl) and tunneled subcutaneously to the back of the neck, exteriorized and secured. Bupivacaine (1%) was applied topically to the site of incision and animals were allowed to recover for 24 h. On the following day, blood pressure was recorded using a pressure transducer (PD23ID, Gould Statham) and Grass polygraph (Model 79D, Grass Instruments) and the heart rate was measured using a tachograph (Model 7P4G Grass Instruments, MA, USA) continuously for 30–45 min in free-moving conscious rats. After 30–45 min, a blood sample was taken for measurement of renin activity. 2K1C rats with a diastolic blood pressure of > 100 mmHg were used while animals with malignant-phase hypertension, as evidenced by the onset of weight loss, were excluded from the study.

2.2. Measurement of plasma renin activity

Renin-dependent hypertension was verified by determination of plasma renin activity. Blood (1 ml) was collected into a pre-chilled syringe containing EDTA to yield a final concentration of 1 mg/ml. After centrifugation, the plasma was frozen and stored at -20°C until it was assayed. Plasma renin activity was determined as angiotensin I generated under control conditions in which converting enzyme and angiotensinase activity were inhibited by the use of EDTA, dimercaprol and 8-hydroxyquinoline. The amount of generated angiotensin I was measured by radioimmunoassay using a commercial polyclonal antiserum against angiotensin I (Du Pont Canada) and a double-antibody determination system.

2.3. In vitro: perfused isolated mesenteric artery preparation

Each animal was anaesthetized with sodium pentobarbital (35 mg/kg, i.v.). The abdominal cavity was opened and the mesenteric artery was cannulated through an incision at the confluence with the dorsal aorta and then isolated as previously described by McGregor (1965). The mesenteric artery and its branches were flushed with heparinized physiological salt solution, transferred to a warmed organ chamber and perfused with Krebs-bicarbonate (normal Krebs) buffer maintained at 37°C and gassed with 95% O_2-5% CO_2. The Krebs-bicarbonate buffer was of the following composition (in mM): NaCl 120, KCl 4.6, glucose 11, $MgSO_4$ 1.2, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 25.3. The pH of the buffer, following saturation with a 95% O_2-5% CO_2 gas mixture, was 7.4. All experiments were performed in both normal Krebs and/or Cl^--free buffer of the following composition (in mM): C_2H_2COONa 120, C_2H_2COOK 3.5, glucose 11, $MgSO_4$ 1.2, $Ca(C_6H_{11}O_7)_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 25. The perfusion rate was kept constant at 5 ml/min using a Polystaltic Peristaltic Pump (Buchler Instruments). Changes in perfusion pressure were measured and recorded using a pressure transducer (PD23ID, Gould Statham) and Grass polygraph (Model 79D, Grass Instruments). The perfused blood vessels were allowed to stabilize for 1 h before the start of each experiment.

2.4. Experimental protocols

The effects of vehicle or niflumic acid on the vasoconstrictor responses to cirazoline in perfused mesenteric vascular beds were characterized in two series of experiments from both 2K1C and sham rats.

2.4.1. Series 1

This procedure was performed using normal Krebs buffer. The tissues were initially exposed to a submaximal dose of cirazoline (9 nmol) and then allowed to equilibrate for an additional hour. A control dose–response curve for cirazoline was constructed from 6 separate bolus injections of cirazoline (0.09–30 nmol). Perfusion pressure was allowed to return to baseline before the bolus injection of

each dose of agonist. Second and third dose–response curves to cirazoline were constructed in the presence of vehicle (0.03 and 0.1% alcohol) and niflumic acid (3 and 10 μM) in the perfusion media. Blood vessels were perfused with buffer containing either vehicle or niflumic acid for 20 min and thereafter dose–response curves for the agonist were constructed. After the completion of each dose–response curve for cirazoline, a single bolus injection of KCl (60 μmol) was also made.

2.4.2. Series 2

The effects of vehicle and niflumic acid were also evaluated in Cl^--free buffer. A control dose–response curve to cirazoline was obtained from mesenteric arteries perfused with normal Krebs as previously described. The tissues were then allowed to stabilize for 40 min and perfused with normal Krebs solution. The solution was then changed to Cl^--free buffer and 20 min was permitted to elapse before a dose–response curve to cirazoline was constructed. After completion of the dose–response curve, the tissues were perfused with normal Krebs for 40 min. Subsequently, the solution was again changed to Cl^--free buffer and blood vessels were perfused with this solution for 20 min and thereafter before the construction of the final dose–response curve to cirazoline. The third dose–response curve to cirazoline was constructed in the presence of either vehicle (0.1% alcohol) or niflumic acid (3 or 10 μM). Separate tissues were used for different concentrations of niflumic acid.

2.5. In vivo: blood flow and vascular conductance

2.5.1. Surgical preparation

An additional group of animals were anaesthetized with sodium pentobarbital (35 mg/kg, i.v.) and an additional catheter (polyethylene tubing I.D. 0.58 mm, O.D. 0.965 mm) was inserted into the right femoral vein for administration of cirazoline. The abdominal cavity was opened through a ventral midline incision and the superior mesenteric artery was exposed and dissected free. A transonic flow probe (Model 1RB630, Transonic Systems) was placed on the mesenteric artery and blood flow measured using a flowmeter (Model T206, Transonic Systems) and displayed on a Grass polygraph (Model 79D, Grass Instruments). Blood pressure and heart rate were continuously monitored. Body temperature in these animals was maintained at 36 ± 1°C using a heating lamp and monitored by a rectal mercury thermometer. After completion of surgery, each animal was allowed to stabilize for a period of 60 min.

2.6. Experimental protocol

The effects of niflumic acid on blood pressure, blood flow and mesenteric vascular conductance were examined in four groups of rats. Each animal initially received a cumulative continuous infusion of cirazoline (0.13, 0.34, 1.00 and 2.77 μg/kg per min), with each dose infused for 6 min. After completion of the first dose–response curve, each animal was allowed to recover for 50 min. This period was sufficient to allow blood pressure, heart rate and mesenteric blood flow to return to the baseline. Each animal then received either vehicle (0.3 ml/kg; $NaHCO_3$ in glucose solution) or niflumic acid (3 mg/kg) as a bolus i.v. injection, and 10 min was allowed to elapse before the second cumulative dose–response curve to cirazoline was constructed.

2.7. Chemicals

All chemicals were purchased from Fisher Scientific (Richmond, B.C., Canada), Sigma (St. Louis, MO, USA) or Research Biochemical International (Natick, MA, USA). Angiotensin I [^{125}I] radioimmunoassay kits was purchased from Du Pont (Mississauga, Ont., Canada). A stock solution of niflumic acid (0.1 M) was prepared in 100% ethanol and diluted to the required concentration in perfusate reservoir for experiments in the isolated mesenteric vascular bed. Niflumic acid was dissolved in $NaHCO_3$ (0.4 M, pH 8.5) with 5% glucose and prepared as stock (10 mg/ml) for in vivo studies. The solutions of niflumic acid were made fresh each day. Cirazoline was made in normal saline (0.9% NaCl) or twice distilled water for in vivo or in vitro studies, respectively.

2.8. Statistical analysis

For the in vitro study, the absolute increases in perfusion pressure following bolus injection of each dose of cirazoline were plotted. Vascular conductance, in vivo, was calculated as flow divided by mean blood pressure (MAP) (MAP was calculated as diastolic blood pressure + 1/3(systolic blood pressure − diastolic blood pressure)). The decreases in conductance were expressed as decreases in percentage of the control conductance obtained just before infusion of cirazoline. All data are presented as mean ± S.E.M. Student's unpaired *t*-test was used for comparisons between two means and two-way analysis of variance (ANOVA) was used for multiple comparisons between the two groups of rats (i.e., 2K1C and sham). Duncan's multiple range test was used to compare between multiple means. $P < 0.05$ was considered significant in the analysis.

3. Results

Systolic and diastolic blood pressure and heart rate of conscious 2K1C rats were significantly ($n = 42$; $P < 0.05$) higher than those of sham rats (Table 1). Furthermore, the plasma renin activity was significantly ($n = 42$; $P < 0.05$) elevated in 2K1C hypertensive rats when compared to that of sham normotensive rats (Table 1).

Table 1
Blood pressure (mmHg), heart rate (beats/min), plasma renin activity (mg/ml per h) and body weight (g) of 2K1C hypertensive and sham normotensive rats

	2K1C	Sham
Arterial pressure		
Systolic	244 ± 5 [a]	134 ± 2
Diastolic	166 ± 4 [a]	94 ± 2
Heart rate	417 ± 8 [a]	370 ± 5
Plasma renin activity	18.37 ± 2.10 [a]	3.03 ± 0.28
Body weight	367 ± 6	392 ± 6

Values are pooled and shown as mean ± S.E.M., $n = 42$ for each group of rats.
[a] Significantly different from sham, $P < 0.05$.

3.1. Effect of niflumic acid on cirazoline-induced vasoconstriction in isolated mesenteric beds perfused with normal Krebs

The basal perfusion pressures in isolated mesenteric arteries of 2K1C hypertensive and sham normotensive rats, perfused with normal Krebs, were 27.4 ± 0.9 and 27.9 ± 0.9 mmHg ($n = 12$), respectively. There were no significant differences between basal perfusion pressures of 2K1C hypertensive versus sham normotensive rats. Bolus injections of cirazoline (0.09–30 nmol) evoked dose-dependent pressor responses in isolated mesenteric arteries from 2K1C hypertensive and sham normotensive rats. Cirazoline-evoked increases in perfusion pressure in mesenteric arteries obtained from 2K1C hypertensive rats were significantly higher than those corresponding in sham normotensive rats (Figs. 1–3). The presence of vehicle (0.03% and 0.1% alcohol) did not influence the dose–response curve to cirazoline (data not shown). The presence of niflumic acid (3 and 10 μM) in the perfusion medium inhibited the vasoconstrictor actions of cirazoline (Fig. 1). Cirazoline-mediated vasoconstriction was significantly ($n = 6$; $P < 0.05$) inhibited at all doses (0.09–30 nmol) in the presence of the higher concentration of niflumic acid, and only at one dose (0.9 nmol) in the presence of the lower concentration of niflumic acid in tissues obtained from either 2K1C or sham rats. The higher concentration of niflumic acid decreased perfusion pressure at 0.09, 0.3, 0.9, 3, 9 and 30 nmol of cirazoline in 2K1C and in sham rats. There were no differences in the magnitude of the reductions caused by the presence of niflumic acid between 2K1C hypertensive and sham normotensive rats. In addition, we found that vasoconstriction evoked by bolus injection of KCl (60 μmol) in isolated mesenteric beds perfused with normal Krebs was not affected by the presence of niflumic acid in the perfusion medium. There was also no difference between the response to KCl in 2K1C hypertensive and sham normotensive rats, 87.5 ± 8.8 and 71.1 ± 9.8 mmHg, respectively.

3.2. Effect of niflumic acid on cirazoline-induced vasoconstriction in isolated mesenteric beds perfused with Cl^--free buffer

We found that when the perfusion buffer was changed from normal Krebs to Cl^--free buffer, there was a transient increase in pressure after which the perfusion pressure stabilized at 28.7 ± 1.1 and 27.3 ± 1.0 mmHg for 2K1C and sham rats, respectively. The transient increase in perfusion pressure in Cl^--free buffer was 18.4 ± 4.4 and 4.9 ± 0.5 mmHg for 2K1C hypertensive and sham normotensive rats, respectively. This increase in perfusion pressure was significantly ($n = 18$; $P < 0.05$) greater in 2K1C hypertensive rats. Cirazoline-induced vasoconstriction in isolated mesenteric beds obtained from 2K1C hypertensive and sham normotensive rats was impaired following perfusion with Cl^--free buffer when compared to normal Krebs (Figs. 2 and 3). The inhibition was significant ($n = 6$; $P < 0.05$) at doses of 3–30 nmol (Fig. 2AB and Fig. 3AB). Perfusion of mesenteric blood vessels with Cl^--free buffer resulted in a significantly ($n = 6$; $P < 0.05$)

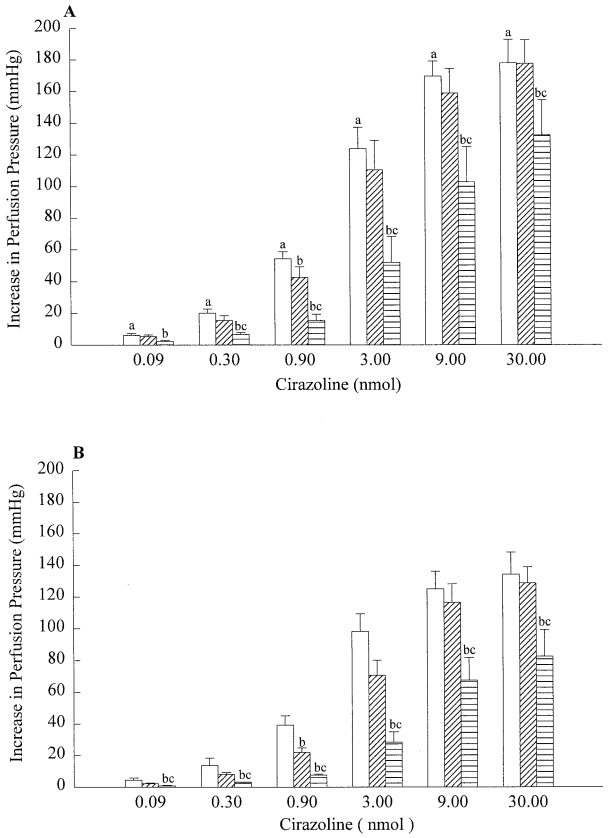

Fig. 1. Concentration–response effect to cirazoline in the absence (open columns) and presence of niflumic acid (3 μM, cross-hatched columns; 10 μM, horizontally hatched columns) in isolated mesenteric arterial beds obtained from 2K1C hypertensive (A) or sham normotensive (B) rats, perfused with normal Krebs at constant flow. Data represent the mean of six experiments ± S.E.M. [a] Significantly different from sham rats, $P < 0.05$ (two-way ANOVA); [b] significantly different from normal Krebs alone, $P < 0.05$; [c] significantly different from niflumic acid (3 μM), $P < 0.05$; (two-way ANOVA followed by Duncan's test).

greater inhibition of cirazoline-mediated vasoconstriction in sham normotensive rats than in 2K1C hypertensive rats (Figs. 2 and 3 inset). We did find that in Cl^--free buffer, cirazoline-mediated vasoconstriction was further inhibited by the presence of niflumic acid in the perfusion media (Figs. 2 and 3). Niflumic acid at the lower concentration significantly ($n = 6$; $P < 0.05$) inhibited cirazoline-mediated vasoconstriction at doses of 0.3, 3, 9 and 30 nmol in 2K1C hypertensive and sham normotensive rats. The magnitude of blockade of cirazoline-mediated vasoconstriction produced by niflumic acid was significantly ($n = 6$; $P < 0.05$) greater in sham rats than in 2K1C rats (Fig. 2 inset). The higher concentration of niflumic acid suppressed responses to cirazoline in Cl^--free buffer in a similar manner (Fig. 3). The presence of a higher concentration of niflumic acid significantly ($n = 6$; $P < 0.05$) inhibited cirazoline-mediated vasoconstriction at doses of 0.9, 3, 9 and 30 nmol in 2K1C hypertensive and in normotensive rats. The magnitude of reduction in vasoconstrictor response to cirazoline was again significantly ($n = 6$; $P < 0.05$) greater in sham normotensive rats than in 2K1C hypertensive rats (Fig. 3 inset). We should add that a repetition of the dose–response curve to cirazoline using Cl^--free buffer in the presence of vehicle was not significantly different from the first dose–response curve to cirazoline in Cl^--free buffer in perfused mesenteric beds (data not shown).

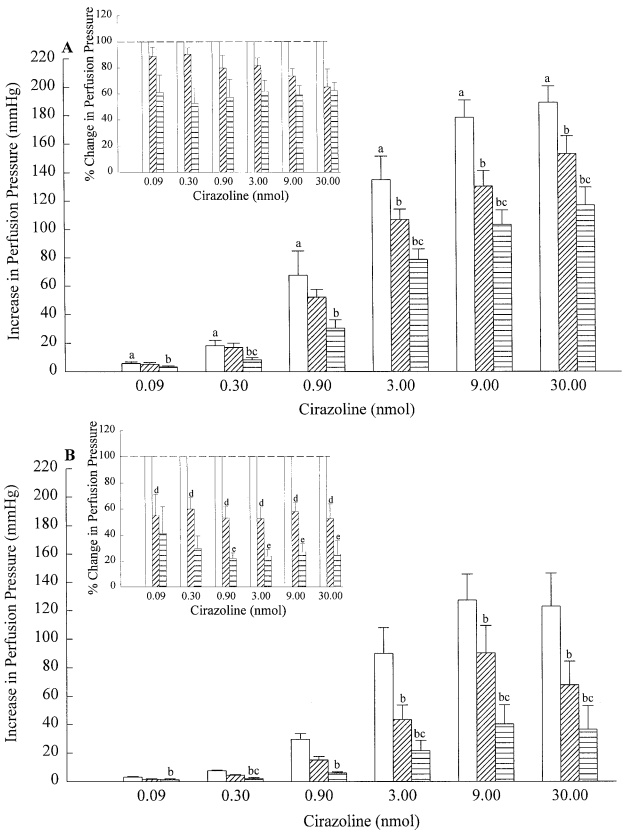

Fig. 2. Concentration–response effect to cirazoline in isolated mesenteric arterial beds obtained from 2K1C hypertensive (A) or sham normotensive (B) rats, perfused with normal Krebs (open columns) and with Cl^--free buffer in the absence of niflumic acid (cross-hatched columns) and with Cl^--free buffer plus niflumic acid (3 μM; horizontally hatched columns) at constant flow. Data represent the mean of six experiments ± S.E.M. [a] Significantly different from sham rats, $P < 0.05$ (two-way ANOVA); [b] significantly different from normal Krebs, $P < 0.05$; [c] significantly different from Cl^--free buffer, $P < 0.05$ (two-way ANOVA followed by Duncan's test). Insert: Percentage change in perfusion pressure corresponding to the data in (A) or (B). [d] Significantly different from Cl^--free buffer in 2K1C hypertensive rats, $P < 0.05$; [e] significantly different from niflumic acid (3 μM) in 2K1C hypertensive rats, $P < 0.05$ (unpaired *t*-test).

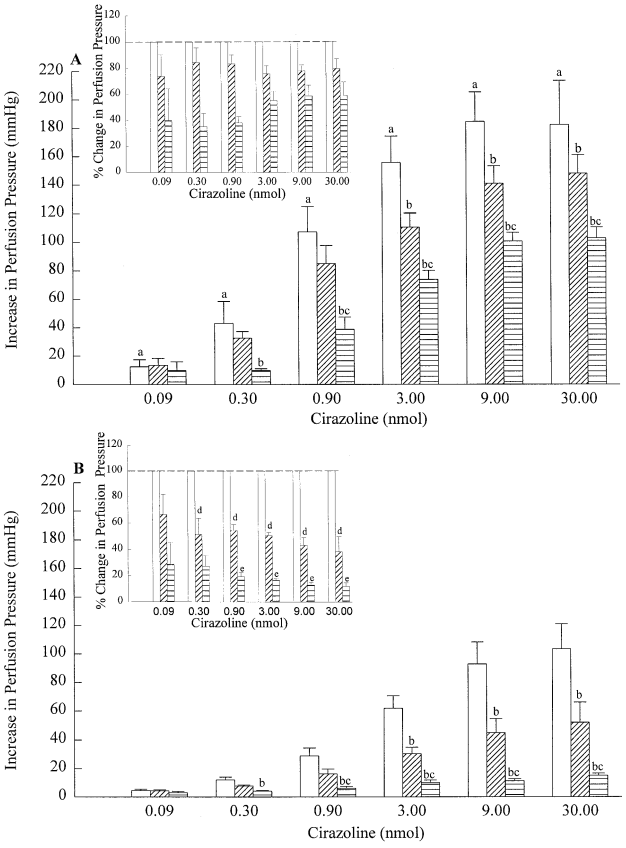

Fig. 3. Concentration–response effect to cirazoline in isolated mesenteric arterial beds obtained from 2K1C hypertensive (A) or sham normotensive (B) rats, perfused with normal Krebs (open columns) and with Cl^--free buffer in the absence of niflumic acid (cross-hatched columns), with Cl^--free buffer plus niflumic acid (10 μM; horizontally hatched columns) at constant flow. Data represent the mean of six experiments ± S.E.M. [a] Significantly different from sham rats, $P < 0.05$ (two-way ANOVA); [b] significantly different from normal Krebs, $P < 0.05$; [c] significantly different from Cl^--free buffer, $P < 0.05$ (two-way ANOVA followed by Duncan's test). Insert: Percentage change in perfusion pressure corresponding to the data in (A) or (B). [d] Significantly different from Cl^--free buffer in 2K1C hypertensive rats, $P < 0.05$; [e] significantly different from niflumic acid (10 μM) in 2K1C hypertensive rats, $P < 0.05$ (unpaired *t*-test).

3.3. Influence of niflumic acid on cirazoline-induced changes on mesenteric vascular conductance in anaesthetized 2K1C hypertensive and normotensive rats

We found no significant difference between the baseline values of superior mesenteric blood flow between 2K1C

Table 2
Mean blood pressure (MAP; mmHg), superior mesenteric artery blood flow (SMAF; ml/min) and conductance (SMAC; ml/mmHg per min) values before and after injection of vehicle ($NaHCO_3$, 0.3 ml/kg) or niflumic acid (3 mg/kg) in 2K1C hypertensive and sham normotensive rats

	MAP		SMAF		SMAC	
	2K1C	Sham	2K1C	Sham	2K1C	Sham
Pre-vehicle	164 ± 14 [a]	98 ± 3	13.9 ± 0.9	14.1 ± 2.5	0.088 ± 0.012 [a]	0.140 ± 0.028
Post-vehicle	148 ± 8 [a]	92 ± 3	14.2 ± 0.9	14.5 ± 1.9	0.097 ± 0.009 [a]	0.158 ± 0.021
Pre-niflumic acid	162 ± 13 [a]	97 ± 5	11.9 ± 1.3	15.5 ± 1.8	0.076 ± 0.012 [a]	0.165 ± 0.027
Post-niflumic acid	153 ± 16 [a]	95 ± 7	12.4 ± 2.8	16.6 ± 2.4	0.086 ± 0.025 [a]	0.180 ± 0.032

Each value represents the mean of five experiments ± S.E.M.
[a] Significantly different from sham, $P < 0.05$.

Table 3
Effects of cirazoline on mean arterial pressure (all values in mmHg) in anaesthetized 2K1C hypertensive and sham normotensive rats before (control) and after treatment with either vehicle ($NaHCO_3$, 0.3 ml/kg) or niflumic acid (3 mg/kg)

	Cirazoline (μg/kg per min)			
	0.13	0.34	1.00	2.77
2K1C				
Control	171 ± 16 [a]	181 ± 17 [a]	200 ± 14 [a]	250 ± 19 [a]
Vehicle-treated	160 ± 9	168 ± 10	188 ± 13	243 ± 15
Control	170 ± 13 [a]	183 ± 15 [a]	215 ± 14 [a]	253 ± 5 [a]
Niflumic acid-treated	154 ± 15	163 ± 14	188 ± 14	237 ± 5
Sham				
Control	104 ± 3	108 ± 2	127 ± 4	172 ± 3
Vehicle-treated	96 ± 4	103 ± 3	118 ± 4	173 ± 5
Control	104 ± 4	109 ± 4	126 ± 5	167 ± 3
Niflumic acid-treated	99 ± 7	106 ± 6	125 ± 5	165 ± 2

Each value represents the mean of five experiments ± S.E.M.
[a] Significantly different from sham rats, $P < 0.05$.

hypertensive and sham normotensive rats. However, the basal vascular conductance in the superior mesenteric artery was significantly ($n = 5$; $P < 0.05$) lower in 2K1C hypertensive rats in comparison to sham normotensive rats (Table 2).

Administration of either niflumic acid or vehicle did not alter the baseline values of MAP, superior mesenteric flow and conductance in anaesthetized rats (Table 2). Intravenous infusion of cumulative doses of cirazoline caused dose-dependent increases in MAP (Table 3), and a decrease in superior mesenteric vascular conductance in 2K1C hypertensive and sham normotensive rats (Table 4). How-

Table 4
Effects of cirazoline on decrease in vascular conductance (% of control) in superior mesenteric artery (all values in ml/mmHg per min) in anaesthetized 2K1C hypertensive and sham normotensive rats before (control) and after treatment with either vehicle ($NaHCO_3$, 0.3 ml/kg) or niflumic acid (3 mg/kg)

	Cirazoline (μg/kg per min)			
	0.13	0.34	1.00	2.77
2K1C				
Control	8.9 ± 2.6	20.1 ± 3.8	39.8 ± 5.0	65.3 ± 3.7
Vehicle-treated	9.2 ± 3.8	17.6 ± 3.8	36.2 ± 5.0	64.4 ± 3.9
Control	10.8 ± 2.0	22.4 ± 3.2	46.8 ± 3.5	68.4 ± 3.2
Niflumic acid-treated	1.3 ± 2.0 [a]	2.0 ± 6.0 [a]	24.3 ± 8.0 [a]	61.0 ± 6.5
Sham				
Control	12.4 ± 1.3	23.2 ± 3.1	42.6 ± 4.4	69.3 ± 3.1
Vehicle-treated	10.4 ± 2.2	19.8 ± 3.9	34.6 ± 4.9	69.1 ± 3.1
Control	12.5 ± 1.6	26.4 ± 3.6	47.7 ± 4.1	72.1 ± 4.0
Niflumic acid-treated	3.0 ± 3.0 [a]	13.6 ± 4.1 [a]	34.3 ± 6.2 [a]	67.0 ± 5.0

Each value represents the mean of five experiments ± S.E.M.
[a] Significantly different from before treatment with niflumic acid, $P < 0.05$.

ever, the degree of reduction in the conductance induced by cirazoline in 2K1C hypertensive rats was similar to those in sham normotensive rats (Table 3). Pretreatment with vehicle did not affect cirazoline-induced changes in MAP or conductance when compared to the absence of vehicle (Tables 3 and 4). In addition, in animals that were treated with niflumic acid, cirazoline-mediated pressor responses were not significantly affected when compared to control (Table 3). However, pretreatment with niflumic acid significantly ($n = 5$; $P < 0.05$) impaired cirazoline-mediated decreases in vascular conductance at doses of 0.13–1.00 μg/kg/min in 2K1C hypertensive and normotensive rats (Table 4).

4. Discussion

In the present study, we have shown that chloride ions play an important role in vasoconstrictor responses that are mediated via the stimulation of α_1-adrenoceptors in the rat isolated perfused mesenteric arteries. We also found that niflumic acid, an antagonist of Ca^{2+}-activated Cl^- channels, was capable of inhibiting cirazoline-induced vasoconstriction both in vitro as well as in vivo. In addition, it seems that the role chloride ions play in cirazoline-mediated vasoconstriction is less in blood vessels obtained from 2K1C hypertensive compared to normotensive rats.

Based on the evidence obtained from electrophysiological studies, a role for agonist-induced Ca^{2+}-activated Cl^- channels has been identified in a number of blood vessels, namely, rabbit and rat portal veins (Byrne and Large, 1988a,b; Pacaud et al., 1986), rabbit ear artery (Amédée et al., 1990), human mesenteric artery (Klöckner, 1993) and rabbit coronary artery (Lamb et al., 1994). At present it is believed that in vascular smooth muscle, noradrenaline-mediated Ca^{2+} release from intracellular stores produces an increase in Cl^- conductance leading to changes in membrane potential and the opening of voltage-gated Ca^{2+} channels (Amédée et al., 1990; Pacaud et al., 1992; Hogg et al., 1993). Ca^{2+}-activated Cl^- currents in vascular smooth muscle have been reported to be blocked by drugs such as 4′,4′-diisothiocyanostilbene-2,2-disulfonic acid and niflumic acid (Pacaud et al., 1989; Kokubun et al., 1991). Our current findings indicate that both niflumic acid and removal of Cl^- inhibited α_1-adrenoceptor-mediated vasoconstriction in perfused mesenteric blood vessels. Noradrenaline has been reported to increase Cl^- efflux while producing depolarization in mesenteric arteries of rats without altering either the rate of potassium efflux or sodium influx (Videbæk et al., 1990). Moreover, responses that are mediated via the α_1-adrenoceptors have been shown to be sensitive to the actions of Ca^{2+} channel antagonists (Chen et al., 1996). In the rat mesenteric microvessels, the Ca^{2+}-entry blocker, nitrendipine, was found to reduce noradrenaline-mediated constriction (Chen et al., 1996).

Current evidence in the literature supports the view that vasoconstrictor responses via the activation of α_1-adrenoceptors in rat mesenteric blood vessels are mediated as a result of the activation of α_{1A}-adrenoceptor sub-types (Kong et al., 1994; Williams and Clarke, 1995; Chen et al., 1996). Different sub-types of α_1-adrenoceptors are believed to be present in smooth muscle but a consensus on sub-classification is still being debated. However, at present, based on pharmacological and biochemical evidence, there appears to be at least three different sub-types of the α_1-adrenoceptor that contribute to contractile responses in smooth muscle. The α_{1A}-adrenoceptors have a high affinity for 5-methyl-urapidil (5-methyl-6[[3-[4-(2-methoxyphenyl)-1-piperazinyl]propyl]amino]-1,3-dimethyluracil), WB 4101 (2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane hydrochloride) and phentolamine, and are insensitive to the actions of the alkylating agent, chloroethylclonidine (Lomasney et al., 1991). The α_{1B}-adrenoceptors are inactivated by chloroethylclonidine and have a low affinity for 5-methyl-urapidil, WB 4101 and phentolamine (Schwinn and Lomasney, 1992). The α_{1D}-adrenoceptors have a high affinity for BMY 7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dione dihydrochloride) and lower affinity for chloroethylclonidine, 5-methyl-urapidil and WB 4101 (Ford et al., 1996). Current evidence using rat aorta indicates that cirazoline has a higher affinity for the α_{1A} and α_{1D} sub-types than the α_{1B} sub-type (Buckner et al., 1996). However, a previous study using human cloned α_1-adrenoceptors had indicated that cirazoline had a higher affinity for the α_{1a} sub-type rather than the α_{1b} and α_{1d} sub-types (Horie et al., 1995). Contraction in vascular smooth muscle mediated via the activation of α_1-adrenoceptors is dependent upon both an influx of Ca^{2+} and Ca^{2+} release from intracellular stores (Brown et al., 1984; Chen and Rembold, 1995; Lum Min and Tabrizchi, 1995, 1996). An elevation in the concentration of intracellular Ca^{2+} is believe to produce an increase in Cl^- conductance (Pacaud et al., 1986; Amédée et al., 1990). Based on our present findings, it would seem as though vasoconstriction that is mediated via the stimulation of α_{1A}-adrenoceptors in the mesenteric artery and its branches is, in part, dependent on the presence of chloride ions.

Here we also find that, in Cl^--free solution, niflumic acid was capable of producing an additive effect in inhibiting cirazoline-induced vasoconstriction. There could be two likely possibilities which may explain such a finding. First, it is possible that niflumic acid inhibited the efflux of residual chloride ions that remained inside the vascular smooth muscle cells in Cl^--free buffer, and thus niflumic acid was capable of further inhibiting cirazoline-induced vasoconstriction. However, an alternative explanation is that either propionate or bicarbonate anions made a small contribution to the process of agonist-induced depolarization via efflux through the Cl^- channels, and in the presence of niflumic acid this effect was blocked. Either or

both of these explanations may account for the additive inhibitory actions of niflumic acid in Cl^--free buffer. However, the possibility that niflumic acid was acting in a non-specific manner to inhibit cirazoline-mediated vasoconstriction is unlikely as we found that niflumic did not inhibit KCl-induced vasoconstriction. Additionally, niflumic acid, at the concentration employed in the present study, has also been reported not to inhibit KCl-mediated contraction in rat aorta (Criddle et al., 1996).

Previously, McGregor and Smirk (1968) had reported that mesenteric arteries from renal hypertensive rats (2K1C) exhibited higher vasoconstrictor responses to noradrenaline. In the present study, we found that cirazoline-induced increases in perfusion pressure were greater in blood vessels from hypertensive rats. The higher vasoconstrictor responses observed in renal hypertensive tissues have been suggested to be the result of increased resistance to flow. Significant reductions in external diameter and increased media-to-lumen ratio has been reported to be responsible for increased vascular reactivity in 2K1C hypertensive rats (Deng and Schiffrin, 1991). In the present study, we also find that basal vascular conductance in situ was lower in 2K1C hypertensive rats when compared to sham normotensive rats. Taken together, these observations could be taken as indicating that morphological changes may account for changes in the function of blood vessels in 2K1C hypertensive rats (Bennett and Thurston, 1996; Li et al., 1996). However, morphological difference may not entirely account for altered behavior of blood vessels in 2K1C hypertensive versus normotensive rats since we did not observe a difference in vasoconstrictor responses evoked by KCl in the present study.

From our in vitro and in vivo studies it is apparent that niflumic acid has a similar efficacy at inhibiting cirazoline-mediated vasoconstriction in both normotensive and hypertensive rats. However, this is not the case when chloride ions are replaced with propionate ions. Our present findings indicate that in Cl^--free solution niflumic acid was more effective at inhibiting cirazoline-mediated vasoconstriction in normotensive when compared to hypertensive rats. Moreover, we did find that removal of chloride ions affected cirazoline-induced vasoconstriction to a greater extent in sham than in hypertensive rats. Differences in resting membrane potential in blood vessels from 2K1C hypertensive than those of sham normotensive rats in Cl^--free buffer may have been responsible for the increased ability of niflumic acid to inhibit cirazoline-induced vasoconstriction in sham when compared with 2K1C hypertensive rats. The possibility that adaptive changes in ion permeability of vascular smooth muscle in 2K1C hypertensive rats may have occurred exists. Certainly, lowering chloride ion concentration of physiological salt solution has been found to result in changes in membrane potential in vascular smooth muscle (Van Helden, 1988). Furthermore, Yoshida et al. (1989) had previously reported that an increase in intracellular Ca^{2+} content of vascular smooth muscle in 2K1C hypertensive rabbits does occur. However, the possibility that an increase in the availability of intracellular Ca^{2+} may be responsible for a diminished role for chloride ions during agonist-mediated pharmacomechanical processes in 2K1C hypertensive rats cannot be determined from our present study.

In summary, our current results suggest that chloride ions play a functional role in α_1-adrenoceptor-mediated vasoconstriction in mesenteric blood vessels. Moreover, in isolated blood vessels from hypertensive rats, an altered functional role for chloride ions may account for a diminished role for this anion in the process that involves α_1-adrenoceptor-stimulated vasoconstriction.

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