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A comparative study of the effects of Cl^- channel blockers on mesenteric vascular conductance in anaesthetized rat

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Abstract

There is evidence to suggest that niflumic acid is capable of selectively inhibiting Ca^{2+}-dependent Cl^- channels. Furthermore, it has been demonstrated that niflumic acid is capable of antagonizing contractile responses due to activation of α_1-adrenoceptor in mesenteric vasculature. Here, we have examined the effects of three Cl^- channel blockers, niflumic acid, indanyloxyacetic acid 94 (IAA-94) and diphenylamine-2-carboxylic acid (DPC) on cirazoline-mediated vasoconstriction in mesenteric blood vessel in vivo. Infusion of cirazoline produced a dose-dependent increase in blood pressure, decrease in superior mesenteric blood flow, mesenteric vascular conductance and heart rate. While niflumic acid and IAA-94 did not have any impact on cirazoline-induced changes in blood pressure, DPC accentuated the pressor effect of cirazoline. Neither agent affected cirazoline-mediated reflex reduction in the heart rate. Niflumic acid, IAA-94 and DPC attenuated α_1-adrenoceptor mediated decrease in mesenteric blood flow and vascular conductance. Based on the profile of the actions of these compounds, it may be suggested that IAA-94 did not appear to act as selective inhibitor of Ca^{2+}-activated Cl^- channels when compared to niflumic acid in the mesenteric blood vessels. In addition, while DPC seems to be as effective as niflumic acid in its effects on mesenteric blood vessels, its actions may be attributed to other pharmacological effects.

Keywords: Mesenteric blood vessel; Vascular conductance; Blood flow; Ca^{2+}-activated, Cl^- channel; α_1-Adrenoceptor

1. Introduction

There is evidence to suggest that niflumic acid is capable of selectively inhibiting Ca^{2+}-dependent Cl^- channels (for review, see Large and Wang, 1996). Furthermore, it has been demonstrated that niflumic acid is capable of antagonizing contractile responses due to activation of α_1-adrenoceptor in mesenteric vasculature (He and Tabrizchi, 1997; Criddle et al., 1997). It has been suggested recently that Cl^- plays a critical role in the process of excitation–contraction coupling in smooth muscle (for review, see Chipperfield and Harper, 2000). In vascular smooth muscle, agonist-initiated depolarization is believed to occur, in part, as a result of changes in Cl^- conductance following the activation of Ca^{2+}-dependent Cl^- channels (Byrne and Large, 1988; Hogg et al., 1993). There is certainly evidence in literature that indicates stimulation of α_1-adrenoceptors and subsequent depolarization is the result of Ca^{2+}-activated Cl^- channels. In essence, stimulation of α_1-adrenoceptor results in release of Ca^{2+} from intracellular stores (sarcoplasmic reticulum) which opens the Ca^{2+}-dependent Cl^- channels which leads to depolarization (Pacaud et al., 1992). Subsequently, this leads to the opening of voltage-dependent Ca^{2+} channels and Ca^{2+} influx which is, in part, responsible for vasoconstriction (Klöckner, 1993).

Evidence from our laboratory and others indicate that in the rat isolated perfused mesenteric bed, α_1-adrenoceptor-mediated vasoconstriction is inhibited by putative Cl^- channel antagonist, niflumic acid (He and Tabrizchi, 1997; Criddle et al., 1997). Remillard et al. (2000) have further demonstrated that Ca^{2+}-dependent Cl^- channels play an important role in contraction of rabbit mesenteric arterioles following the stimulation of α_1-adrenoceptors. As well, we have demonstrated that niflumic acid administration affects vascular conductance and blood flow in the mesenteric vascular bed in vivo (He and Tabrizchi, 1997). Collectively, the current evidence in the literature indicates that Cl^- channels play an important role in vasoconstriction involving resistance blood vessels.

We undertook the present investigation to compare the impact of the Cl^- channel antagonist, niflumic acid, to two

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Table 1
Baseline values of mean arterial blood pressure (blood pressure; mm Hg), superior mesenteric arterial blood flow (SMAF; ml/min), heart rate (beats/min), superior mesenteric arterial conductance (SMAC; ml/min Hg per min) values before and after injection of vehicle (0.4 M $NaHCO_3$ in 5% glucose; 1 ml/kg), niflumic acid (NFA; 10 and 30 mg/kg), indanyloxyacetic acid 94 (IAA-94; 3 and 10 mg/kg) and diphenylamine-2-carboxylic acid (DPC; 3 and 10 mg/kg) in anaesthetized rat

Groups		Blood pressure	SMAF	Heart rate	SMAC
Vehicle	Pre	110 ± 4	9.8 ± 1.3	383 ± 5	0.090 ± 0.013
	Post	105 ± 3	8.4 ± 1.2	378 ± 9	0.082 ± 0.014
NFA (10)	Pre	98 ± 4	11.4 ± 1.3	342 ± 7	0.116 ± 0.012
	Post	93 ± 5	10.7 ± 1.3	342 ± 11	0.116 ± 0.014
NFA (30)	Pre	111 ± 4	8.6 ± 0.7	373 ± 16	0.078 ± 0.006
	Post	97 ± 3	7.1 ± 0.8	367 ± 14	0.073 ± 0.006
IAA-94 (3)[n]	Pre	102 ± 4	11.0 ± 1.3	354 ± 15	0.108 ± 0.012
	Post	99 ± 4	10.0 ± 1.4	358 ± 16	0.101 ± 0.013
IAA-94 (10)	Pre	108 ± 4	11.0 ± 1.0	362 ± 8	0.103 ± 0.010
	Post	105 ± 5	9.8 ± 0.9	362 ± 9	0.093 ± 0.009
DPC (3)	Pre	110 ± 4	10.8 ± 1.0	348 ± 5	0.099 ± 0.007
	Post	104 ± 3	10.0 ± 0.9	343 ± 7	0.096 ± 0.007
DPC (10)	Pre	122 ± 2	11.7 ± 1.0	360 ± 9	0.096 ± 0.008
	Post	108 ± 6	8.6 ± 1.0	367 ± 18	0.080 ± 0.008

Each value represents the mean of six ([n]except $n = 8$) experiments ± S.E.M.

other putative Cl^- channel antagonists, indanyloxyacetic acid 94 (IAA-94) and diphenylamine-2-carboxylic acid (DPC) on α_1-adrenoceptor-mediated vasoconstriction in mesenteric blood vessels in vivo. The impact of these drugs on arterial blood pressure, heart rate, mesenteric blood flow and vascular conductance was examined in anaesthetized rats.

2. Materials and methods

2.1. Surgical preparation

Male Sprague–Dawley rats (250–330 g) were anaesthetized with thiobutabarbital (100 mg/kg; i.p.). Catheters (polyethylene tubing I.D. 0.58 mm, O.D. 0.965 mm) were inserted into the left and right femoral veins for administration of drugs and left femoral artery for measurement of arterial blood pressure. The catheters were filled with heparinized saline (25 IU/ml in 0.9% NaCl). The animals were tracheotomized with 14-gauge × 2 in. needle. 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, New York, USA) was placed on the mesenteric artery and blood flow was measured using a flowmeter (Model T106, Transonic Systems) and displayed on a Grass polygraph (Model 7P1F, Grass Instruments, Massachusetts, USA) (Tabrizchi and Pugsley, 2000). Arterial blood pressure was recorded with a pressure transducer (Model TNF-R, Ohmeda, Wisconsin, USA) connected to a polygraph (Model 7P1F, Grass Instruments). Heart rate was derived from the upstroke of the arterial pulse pressure by a tachograph (Model 7P44D, Grass Instruments). Body temperature was maintained at 36 ± 1 °C using a heating lamp and monitored by a rectal thermometer. After completion of surgery, each animal was allowed to stabilize for a period of 60 min while blood pressure, mesenteric blood flow and heart rate were continuously monitored.

2.2. Experimental protocol

Animals were randomly assigned to seven groups ($n = 6–8$ in each group). Following stabilization period,

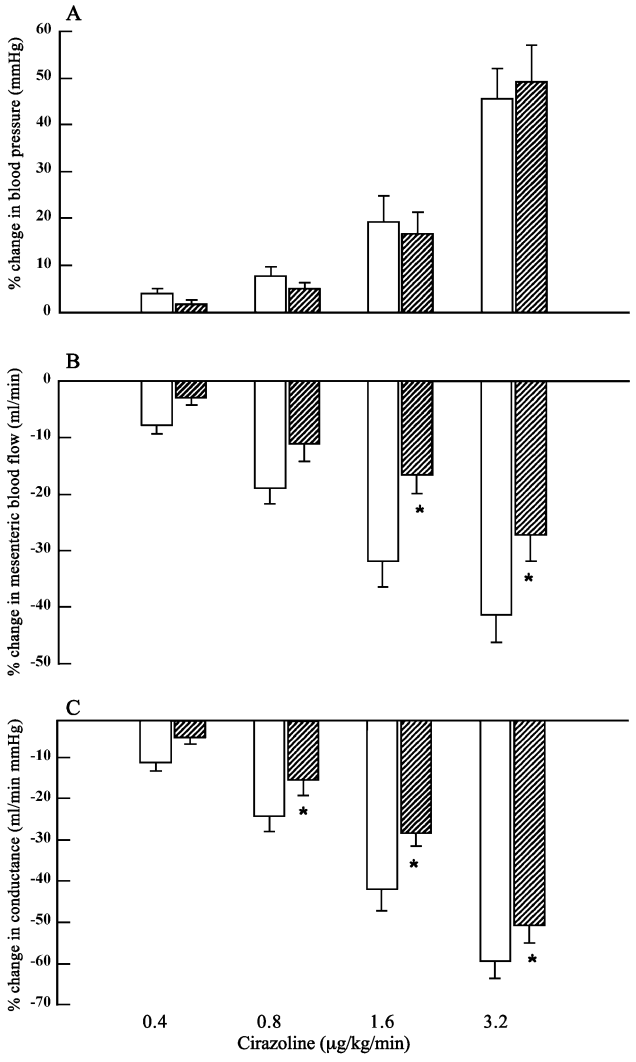

Fig. 1. Concentration–response effect to cirazoline in the absence (opened bars) and presence of niflumic acid (10 mg/kg; hatched bars) on (A) blood pressure (percentage of control prior to infusion of cirazoline), (B) superior mesenteric blood flow (percentage of control prior to infusion of cirazoline) and (C) mesenteric vascular conductance (percentage of control prior to infusion of cirazoline). Each value represents mean ± S.E.M. of six experiments. * Significantly different from respective pre-drug value; $P < 0.05$.

each animal received a cumulative continuous infusion of cirazoline (0.4, 0.8, 1.6 and 3.2 μg/kg per min), with each dose infused for a period of 6 min. After the completion of the first dose–response curve, each animal was allowed to recover for 60 min. This period was sufficient to allow blood pressure, heart rate and mesenteric blood flow to return to baseline (He and Tabrizchi, 1997). Animals in Group I then received a single bolus dose of vehicle (1 ml/kg; 0.4 M $NaHCO_3$ in 5% glucose). Groups II and III received bolus doses of niflumic acid (10 and 30 mg/kg), groups IV and V received IAA-94 (3 and 10 mg/kg) and groups VI and VII received DPC (3 and 10 mg/kg). A period of 15 min was allowed to elapse after the bolus injection of vehicle or drugs before the second cumulative dose–response curve to cirazoline was constructed.

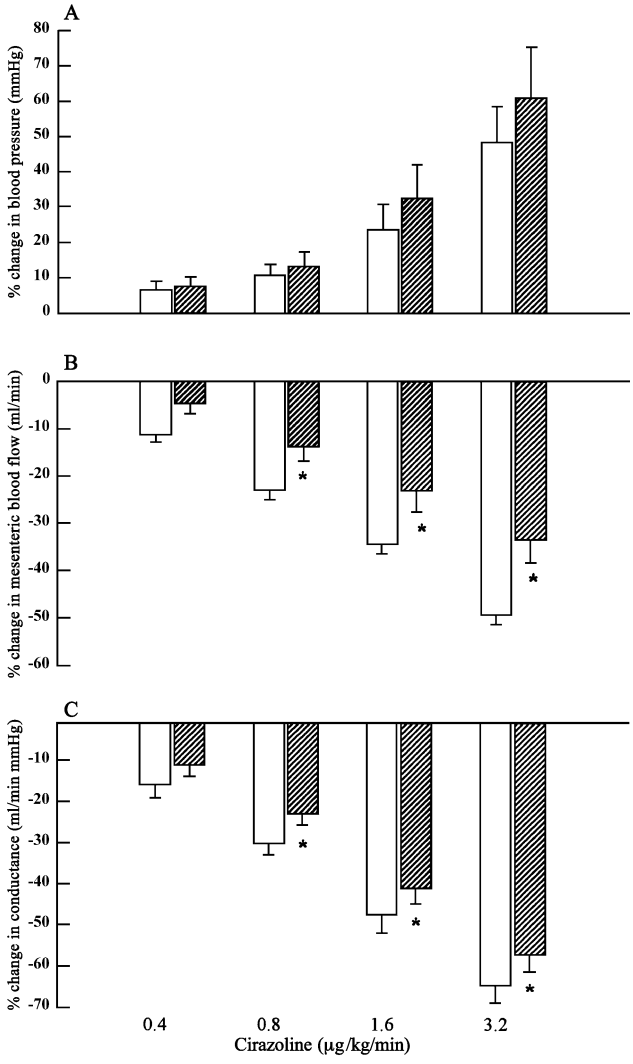

Fig. 2. Concentration–response effect to cirazoline in the absence (opened bars) and presence of niflumic acid (30 mg/kg; hatched bars) on (A) blood pressure (percentage of control prior to infusion of cirazoline), (B) superior mesenteric blood flow (percentage of control prior to infusion of cirazoline) and (C) mesenteric vascular conductance (percentage of control prior to infusion of cirazoline). Each value represents mean ± S.E.M. of six experiments. * Significantly different from respective pre-drug value; $P < 0.05$.

Table 2
Heart rate (beats/min) values in different groups of animals before (Pre) and after (Post) treatment with vehicle (0.4 M $NaHCO_3$ in 5% glucose; 1 ml/kg), niflumic acid (NFA; 10 and 30 mg/kg), indanyloxyacetic acid 94 (IAA-94; 3 and 10 mg/kg) and diphenylamine-2-carboxylic acid (DPC; 3 and 10 mg/kg) during infusion with various doses of cirazoline (μg/kg per min) in anaesthetized rats

Groups		0.4	0.8	1.6	3.2
Vehicle	Pre	378 ± 4	365 ± 7[a]	341 ± 9[a,b,c]	322 ± 7[a,b,c,d]
	Post	375 ± 10	362 ± 9	338 ± 10[a,b,c]	320 ± 9[a,b,c,d]
NFA (10)	Pre	340 ± 8	325 ± 13	298 ± 10[a,b,c]	282 ± 8[a,b,c]
	Post	339 ± 10	332 ± 9	307 ± 10[a,b,c]	292 ± 10[a,b,c]
NFA (30)	Pre	365 ± 15	345 ± 17[a,b]	323 ± 13[a,b,c]	315 ± 12[a,b,c]
	Post	367 ± 13	353 ± 14	335 ± 12[a,b]	325 ± 11[a,b,c]
IAA-94 (3)[n]	Pre	351 ± 12	343 ± 12	328 ± 10[a,b,c]	311 ± 8[a,b,c,d]
	Post	355 ± 15	346 ± 13	334 ± 11[a,b]	316 ± 9[a,b,c,d]
IAA-94 (10)	Pre	353 ± 8	345 ± 9[a]	330 ± 9[a,b,c]	317 ± 7[a,b,c,d]
	Post	357 ± 8	348 ± 9[a]	337 ± 8[a,b,c]	320 ± 6[a,b,c,d]
DPC (3)	Pre	345 ± 4	335 ± 4	312 ± 3[a,b,c]	297 ± 4[a,b,c,d]
	Post	343 ± 7	335 ± 6	315 ± 4[a,b,c]	298 ± 5[a,b,c,d]
DPC (10)	Pre	355 ± 9	343 ± 9	330 ± 8	318 ± 7[a,b]
	Post	363 ± 17	362 ± 19	348 ± 17	333 ± 13[a]

Each value represents the mean of six experiments ([n]except $n = 8$) ± S.E.M.
[a] Significantly different from the control value.
[b] Significantly different from 0.4 μg/kg per min dose of cirazoline.
[c] Significantly different from 0.8 μg/kg per min dose of cirazoline.
[d] Significantly different from 1.6 μg/kg per min dose of cirazoline.

2.3. Chemicals

Cirazoline, niflumic acid, indanyloxyacetic acid 94 (IAA-94) and diphenylamine-2-carboxylic acid (DPC) were purchased from Sigma/Research Biochemical International (Oakville, Ontario, Canada). The three Cl^- channel antagonists were dissolved in 0.4 M $NaHCO_3$ in 5% glucose and cirazoline was dissolved in 0.9% NaCl.

2.4. Data and statistical analysis

Vascular conductance was calculated as flow divided by mean arterial blood pressure (mean arterial blood pressure = 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 prior to the infusion of cirazoline. Vascular conductance is considered to be the most appropriate index for vasculature if the changes in vascular tone result primarily in changes in blood flow rather than pressure (Lautt, 1989). All data are presented as mean ± S.E.M. One-way analysis of variance (ANOVA) was used for comparison. Duncan's multiple range test was used to compare between multiple means. In all cases, a probability of error less than 0.05 was selected as the criterion for statistical significance.

3. Results

There were no significant differences in the baseline values of mean arterial blood pressure, heart rate, superior mesenteric blood flow and mesenteric vascular conductance among the various groups of rats (Table 1). Intravenous infusion of cirazoline (0.4, 0.8, 1.6 and 3.2 μg/kg per min) caused a dose-dependent increase in mean arterial blood pressure, decrease in heart rate, mesenteric blood flow and mesenteric vascular conductance in all seven groups. Administration of vehicle did not significantly affect cirazoline-induced changes in mean arterial blood pressure, heart rate, mesenteric blood flow or mesenteric vascular conductance when compared to the effects of cirazoline prior to the administration of vehicle (Table 1). The baseline values after the administration of Cl^- channel antagonists at either dose level were not significantly different when compared to the vehicle-treated group (Table 1).

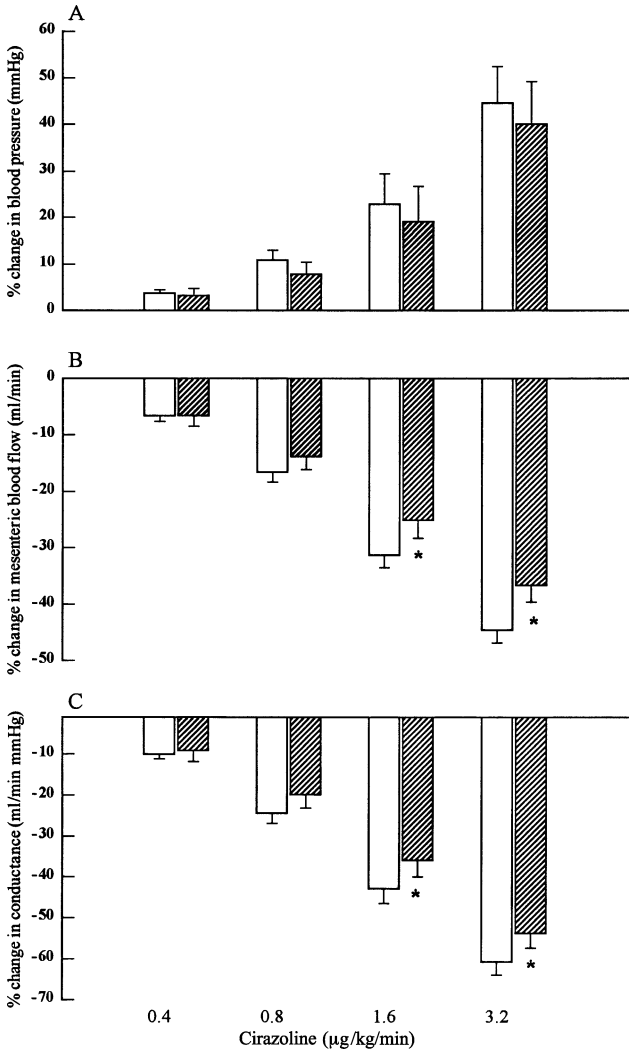

Fig. 3. Concentration–response effect to cirazoline in the absence (opened bars) and presence of IAA-94 (3 mg/kg; hatched bars) on (A) blood pressure (percentage of control prior to infusion of cirazoline), (B) superior mesenteric blood flow (percentage of control prior to infusion of cirazoline) and (C) mesenteric vascular conductance (percentage of control prior to infusion of cirazoline). Each value represents mean ± S.E.M. of eight experiments. * Significantly different from respective pre-drug value; $P < 0.05$.

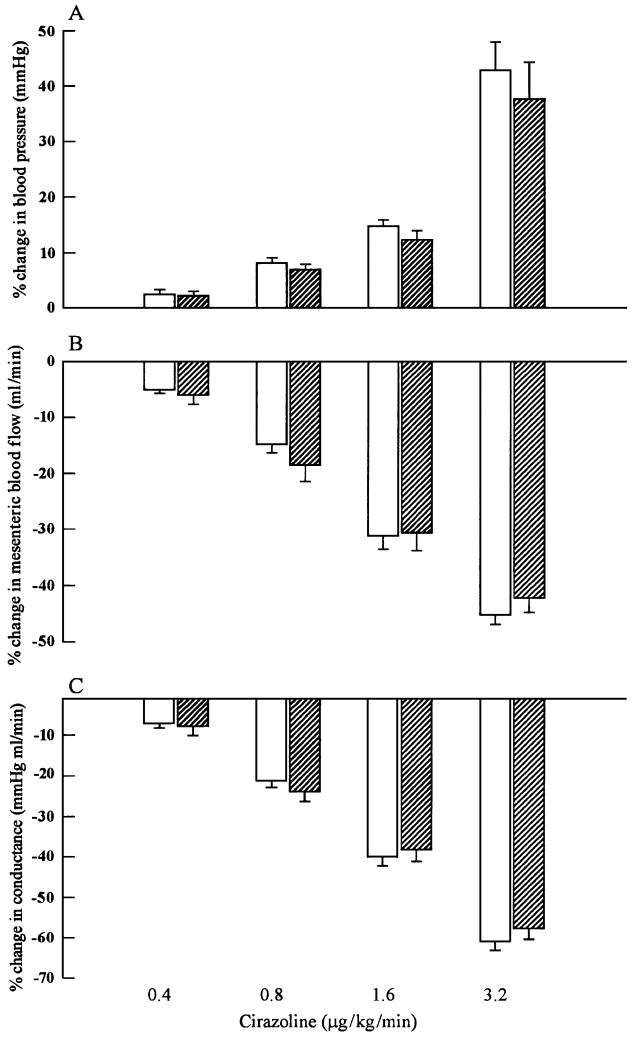

Fig. 4. Concentration–response effect to cirazoline in the absence (opened bars) and presence of IAA-94 (10 mg/kg; hatched bars) on (A) blood pressure (percentage of control prior to infusion of cirazoline), (B) superior mesenteric blood flow (percentage of control prior to infusion of cirazoline) and (C) mesenteric vascular conductance (percentage of control prior to infusion of cirazoline). Each value represents mean ± S.E.M. of six experiments. * Significantly different from respective pre-drug value; $P < 0.05$.

3.1. Effects of niflumic acid on vascular effects of cirazoline in mesenteric bed

Bolus intravenous injections of niflumic acid (10 and 30 mg/kg) did not affect cirazoline-mediated increases in mean arterial blood pressure or decreases in heart rate when compared to control (Figs. 1A, 2A and Table 2). However, niflumic acid at the lower dose, significantly attenuated cirazoline-mediated decreases in mesenteric blood flow at the two higher doses of α_1-adrenoceptor agonist (Fig. 1B).

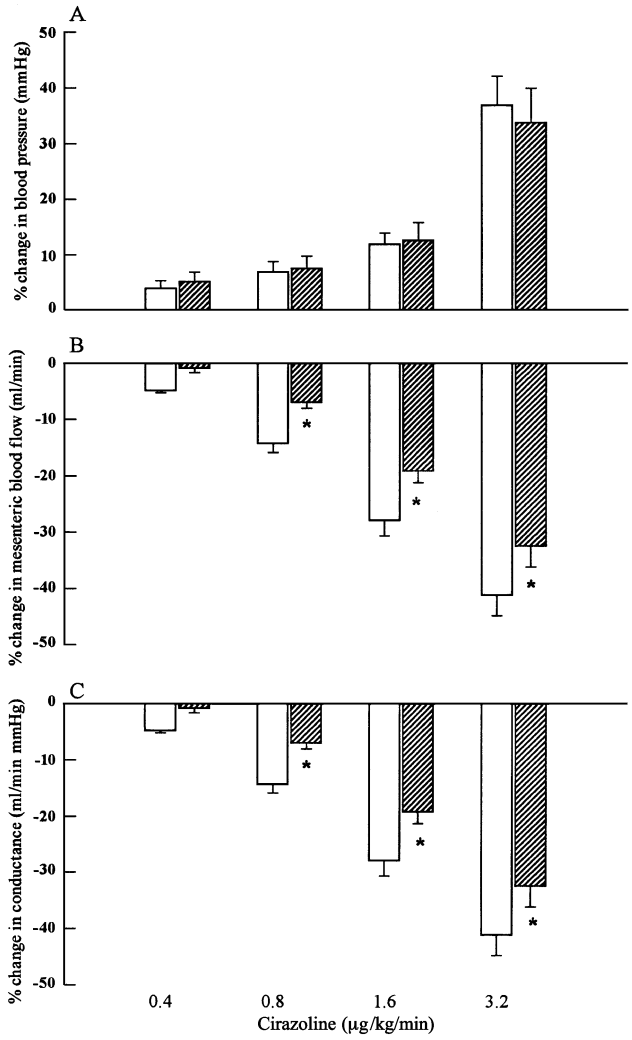

Fig. 5. Concentration–response effect to cirazoline in the absence (opened bars) and presence of DPC (3 mg/kg; hatched bars) on (A) blood pressure (percentage of control prior to infusion of cirazoline), (B) superior mesenteric blood flow (percentage of control prior to infusion of cirazoline) and (C) mesenteric vascular conductance (percentage of control prior to infusion of cirazoline). Each value represents mean ± S.E.M. of six experiments. * Significantly different from respective pre-drug value; $P < 0.05$.

At the higher dose, niflumic acid significantly attenuated cirazoline-induced vasoconstriction at the three higher doses of α_1-adrenoceptor agonist when compared to the control (Fig. 2B). Moreover, pretreatment with niflumic acid (at both doses) inhibited cirazoline-mediated decreases in mesenteric vascular conductance. The effects were significant at the three higher administered doses of α_1-adrenoceptor agonist (Figs. 1C and 2C).

3.2. Effects of IAA-94 on vascular effects of cirazoline in mesenteric bed

Bolus intravenous injections of IAA-94 (3 and 10 mg/kg) did not affect cirazoline-mediated responses on mean arterial blood pressure or heart rate (Figs. 3A, 4A and Table 2). However, IAA-94 at the lower dose significantly attenuated cirazoline-mediated decreases in mesenteric blood flow and mesenteric vascular conductance when compared to the control curve. The effects were significant at the higher two doses of cirazoline (Fig. 3B and 3C). In contrast, higher dose of IAA-94 did not have any effect on mesenteric blood flow and mesenteric vascular conductance (Fig. 4B and 4C).

3.3. Effects of DPC on vascular effects of cirazoline in mesenteric bed

Bolus intravenous injections of DPC (3 and 10 mg/kg) did not affect cirazoline-mediated decreases in heart rate

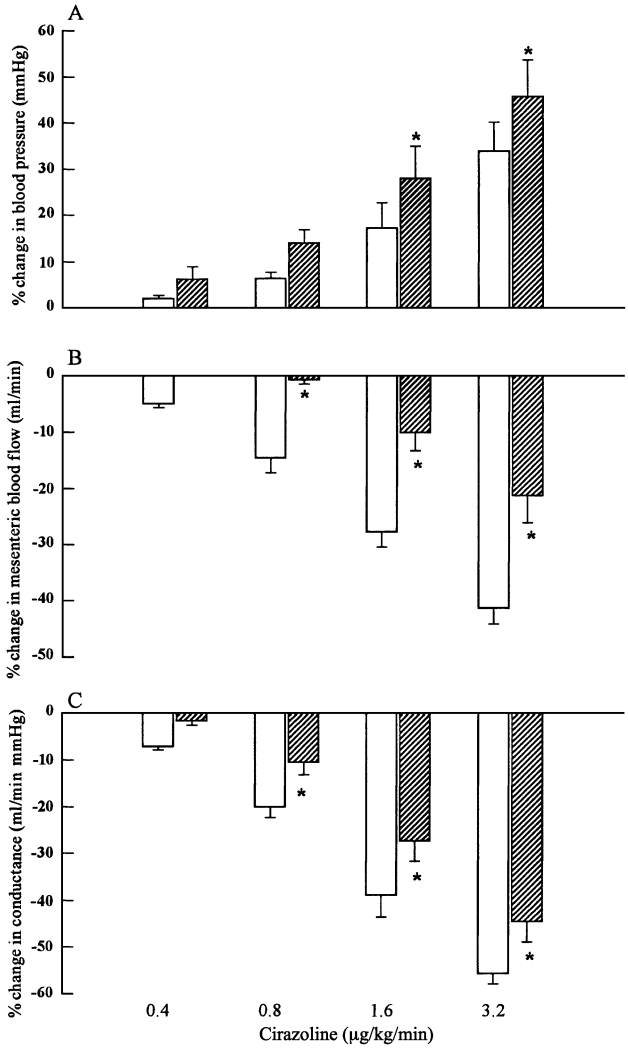

Fig. 6. Concentration–response effect to cirazoline in the absence (opened bars) and presence of DPC (10 mg/kg; hatched bars) on (A) blood pressure (percentage of control prior to infusion of cirazoline), (B) superior mesenteric blood flow (percentage of control prior to infusion of cirazoline) and (C) mesenteric vascular conductance (percentage of control prior to infusion of cirazoline). Each value represents mean ± S.E.M. of six experiments. * Significantly different from respective pre-drug value; $P < 0.05$.

(Table 2). Pretreatment with DPC (at the lower dose) did not affect cirazoline-mediated increases in mean arterial blood pressure (Fig. 5A). However, DPC at the higher dose level did significantly accentuate the pressor responses to cirazoline at the two higher doses (Fig. 6A). Moreover, administration of DPC at both dose levels attenuated cirazoline-mediated decreases in the mesenteric blood flow. The impact of DPC was significant at the higher doses of cirazoline when compared to the effect of α_1-adrenoceptor agonist in the absence of DPC (Figs. 5B and 6B). Pretreatment with DPC also significantly attenuated cirazoline-mediated decreases in mesenteric vascular conductance at the three higher doses of α_1-adrenoceptor agonist (Figs. 5C and 6C).

4. Discussion

In the present investigation, we find that administration of three putative Cl^- channel antagonists, niflumic acid, IAA-94 or DPC, did not have a significant impact on basal mean arterial blood pressure, heart rate, mesenteric blood flow, or mesenteric vascular conductance in thiobutabarbital-anaesthetized rats. However, we did find that all three antagonists impaired cirazoline-induced reduction in mesenteric blood flow and mesenteric vascular conductance without significantly impairing cirazoline-mediated increases in blood pressure or decreases in heart rate.

It is recognized that among the various Cl^- channel antagonists, niflumic acid appears to be the most potent and selective inhibitor of Ca^{2+}-dependent Cl^- channels (for review, see Large and Wang, 1996). There is evidence to suggest that while niflumic acid is capable of selectively inhibiting Ca^{2+}-dependent Cl^- channels by blocking open state of Cl^- channels at concentrations of up to 50 μM, it does not appear to have any effect on K^+ currents (Hogg et al., 1994). Based on the evidence currently available in the literature, niflumic acid was found to produce a concentration-dependent and reversible inhibition of noradrenaline-evoked contractions in the rat aorta (Criddle et al., 1996), as well as, those of 5-hydroxytryptamine-mediated contractions in the rat stomach fundus (Scarparo et al., 2000). In our laboratory, we have demonstrated that niflumic acid is capable of inhibiting cirazoline-induced vasoconstriction in rat isolated perfused mesenteric arteries from 2K1C hypertensive and sham normotensive rats (He and Tabrizchi, 1997). In addition, it has been reported that niflumic acid is capable of inhibiting noradrenaline-induced and 5-hydroxytryptamine-induced vasoconstriction in the rat isolated perfused mesenteric vascular bed (Criddle et al., 1997). Furthermore, inhibition of agonist-mediated vasoconstriction in rat isolated perfused mesenteric arteries produced by the dihydropyridine Ca^{2+} channel antagonist, nifedipine, was not reportedly additive with the inhibitory effects of niflumic acid. Thus, leading to the suggestion that the resultant inhibition in blood vessels produced by niflumic acid ultimately occurred from an impairment in the activation of voltage-gated Ca^{2+} channel (Criddle et al., 1997). More recently, Remillard et al. (2000) have also shown that niflumic acid reversed the contraction produced by the selective α_1-adrenoceptor agonist, phenylephrine, in rabbit mesenteric resistance arteries. Taken together, it would appear that niflumic acid is capable of inhibiting receptor-mediated contraction/vasoconstriction by affecting Ca^{2+}-dependent Cl^- channels. In the present experiment, we have found that niflumic acid does not have any effect on the pressor effects of cirazoline. This is consistent with our previous findings in normotensive and hypertensive animals (He and Tabrizchi, 1997). In the latter study, we found treatment of animals with niflumic acid (3 mg/kg) did not have any effect on the pressor actions of cirazoline but did attenuate the effects of cirazoline on mesenteric vascular conductance (He and Tabrizchi, 1997). Here, it is also apparent that niflumic acid in a dose-dependent manner inhibited α_1-adrenoceptor-mediated decrease in mesenteric blood flow and mesenteric vascular conductance.

IAA-94 is a relatively selective antagonist of Cl^- channels in the epithelial cells (Landry et al., 1987). In addition, it has been demonstrated that IAA-94 inhibited Ca^{2+}-dependent Cl^- currents in rabbit portal vein smooth muscle cells, where it was suggested that it may interact with the open Cl^- channels (Greenwood et al., 1995). In contrast, experiments carried out by Baron et al. (1991) have revealed that in rat portal vein cells, IAA-94 was ineffective on Ca^{2+}-dependent Cl^- currents, although other Cl^- channel blockers (diisothiocyanostilbene-2,2′-disulfonic acid, anthracene-9-carboxylic acid and diphenylamine-2,2′-dicarboxylic acid) were quite effective in inhibiting Ca^{2+}-dependent Cl^- currents in the same preparation.

IAA-94 has also been reported to be capable of inhibiting myogenic vasoconstriction in isolated cerebral blood vessels without affecting K^+-induced vasoconstriction (Nelson et al., 1997). Moreover, in rabbit portal vein cells, IAA-94 was found to inhibit hypotonicity-activated Cl^- current, which is activated as a consequence of cell swelling (Greenwood and Large, 1998). However, there is also evidence that IAA-94 can block L-type Ca^{2+} currents in rat cerebral arteries (Doughty et al., 1998). Evidence from our laboratory has revealed that while IAA-94 (<30 μM) inhibits cirazoline-mediated contractions in aortic rings from normal, as well as, Dahl salt-sensitive hypertensive and salt-resistant normotensive rats, it does not affect KCl-induced contractions in the same preparation (Duggan and Tabrizchi, 2000; Tabrizchi and Duggan, 2000). In our present experiment, we find that IAA-94 did not have any effect on cirazoline-induced increases in arterial blood pressure and decreases in heart rate. The absence of dose-dependent inhibition by the higher dose of IAA-94 observed in our present investigation may be indicative of the fact that IAA-94 had some other effect which counteracted its Cl^- channel blocking effects. In comparison to niflumic acid, IAA-94 appears to be less effective in

inhibiting cirazoline-mediated vasoconstriction in mesenteric vascular bed. This may be attributed to the fact that IAA-94 is less potent and/or less selective against Ca^{2+}-dependent Cl^- channels. Alternatively, another plausible explanation could be that IAA-94 has additional cardiovascular effects that are not shared by niflumic acid.

DPC is also recognized as a blocker of Cl^- channels (Di Stefano et al., 1985; Gogelein, 1988; Conte Camerino et al., 1989). Using the whole-cell patch–clamp technique, it has been demonstrated that DPC inhibits Ca^{2+}-activated Cl^- currents in the rat portal vein cells (Baron et al., 1991). DPC has also been reported to selectively block the inward-rectifying K^+ current and the non-selective cation channel in turtle colon epithelial cells without affecting the large conductance K^+ channels (Richards and Dawson, 1993). The whole-cell voltage-clamp experiments have also revealed that DPC is a reversible blocker of fast Na^+ channels, and L-type Ca^{2+} channels in rat ventricular cardiomyocytes (Conforti et al., 1994). DPC has been found to block the cystic fibrosis transmembrane conductance regulator Cl^- currents, and L-type Ca^{2+} currents from isolated guinea pig ventricular myocytes using the patch clamp technique (Walsh and Wang, 1996). Taken together, the evidence indicates that DPC might not be quite a selective Cl^- channel blocker.

DPC has also been found to relax mouse tracheal smooth muscle through inhibition of epithelial Cl^- channels (Fortner et al., 2001). In our present investigation, we find that DPC, at both lower and higher doses attenuated cirazoline-mediated reduction in mesenteric blood flow, as well as, mesenteric vascular conductance. Our present findings indicated that DPC was equally effective as niflumic acid in inhibiting the actions of cirazoline in mesenteric bed. It is certainly possible that the impact of DPC on cirazoline-mediated effects in mesenteric blood vessels may be due to its other pharmacological effects. It was interesting to note that while DPC inhibited cirazoline-mediated vasoconstriction in mesenteric bed, at the same time, it also accentuated the pressor effects of cirazoline. This pharmacological effect of DPC was in contrast to the actions of either niflumic acid or IAA-94, and may thus be attributed to actions of DPC on other sites within the cardiovascular system.

In our present study, it is quite apparent that the administration of these antagonists did not have any effects on basal heart rate nor did they appear to have any impact on cirazoline-induced reflex-mediated reduction in heart rate. This would obviously suggest that none of these compounds had any impact on reflex-mediated bradycardia subsequent to the elevation of mean arterial blood pressure due to the administration of cirazoline.

In conclusion, IAA-94 did not appear to act as selective inhibitor of Ca^{2+}-activated Cl^- channels when compared to niflumic acid in the mesenteric blood vessels. Moreover, while DPC seemed to be as effective as niflumic acid in its effects on mesenteric blood vessels, its actions are most likely attributed to other pharmacological effects.

Acknowledgements

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