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Role of Cl^- channels in α-adrenoceptor-mediated vasoconstriction in the anesthetized rat

Fred S. Lamb [a,*], Neil W. Kooy [a], Stephen J. Lewis [b]

[a] *Department of Pediatrics, 5040-B RCP, The University of Iowa Hospitals, 200 Hawkins Drive, Iowa City, IA, 52242 USA*
[b] *Department of Pharmacology, University of Iowa, Iowa City, IA, USA*

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Abstract

In vitro studies have provided evidence that Cl^- ion currents are important for activation of vascular smooth muscle contraction. The stilbene, 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS), disrupts Cl^- metabolism by blocking Cl^- channels and by inhibiting Cl^- bicarbonate exchange. The aims of this study were to: (i) characterize the hemodynamic responses produced by DIDS in pentobarbital anesthetized rats, and (ii) examine vasoconstrictor responses to norepinephrine before and after administration of DIDS. DIDS (2.5–50 μmol/kg, 92.5 μmol/kg total dose, i.v.) produced dose-dependent but transient reductions in mean arterial blood pressure and in hindquarter, renal and mesenteric vascular resistances. Prior to the administration of DIDS, norepinephrine (1.0–5.0 μg/kg, i.v.) produced dose-dependent increases in mean arterial pressure, renal resistance and mesenteric resistance, but decreases in hindquarter resistance that were inversely related to dose. After administration of DIDS, the peak pressor responses produced by norepinephrine were either slightly diminished (1.0, 2.5 μg/kg) or unchanged (5.0 μg/kg). Peak norepinephrine-induced changes in hindquarter and renal vascular resistance were unaffected by DIDS, while increases in mesenteric resistance were augmented. The total norepinephrine-induced increases in mean arterial pressure (mm Hg × s) were markedly reduced by DIDS. These effects of DIDS on norepinephrine-induced responses were similar, but not identical to those of the voltage-sensitive Ca^{2+} channel blocker, nifedipine (500 nmol/kg, i.v.). These findings suggest that DIDS may interfere with norepinephrine-induced depolarization of resistance arteries, thereby preventing activation of voltage-sensitive Ca^{2+} channels.

Keywords: Cl^- channel; Vasoconstriction; Stilbene derivative; Smooth muscle, vascular

1. Introduction

Disrupting chloride ion (Cl^-) transport or blocking Cl^- channels inhibits contractile responses of isolated blood vessels to norepinephrine (Criddle et al., 1996; Lamb and Barna, 1998a,b). Agonist-induced Cl^- conductances have been characterized in a number of vascular tissues (Large and Wang, 1996) and norepinephrine has been shown to activate a Cl^- conductance in vascular smooth muscle cells from portal vein (Byrne and Large, 1988; Pacaud et al., 1989), mesenteric vein (Van Helden, 1988) and ear artery (Amedee et al., 1990). In addition to their effects on vascular smooth muscle, Cl^- channels may also be important modulators of endothelial calcium (Ca^{2+}) signaling (Himmel et al., 1993; Nilius et al., 1996). An endothelial Cl^- conductance is activated by bradykinin, histamine and ATP, and appears to be important for Ca^{2+} entry (Hosoki and Iijima, 1994; Song and Davis, 1994).

Activation of Cl^- current in vascular smooth muscle produces a change in membrane potential because Cl^- is actively transported inwardly and is not in electrochemical equilibrium at resting membrane potentials. The accumulation of Cl^- is due to the activity of three distinct transport systems including Cl^- bicarbonate exchange, sodium–potassium–$2Cl^-$ co-transport, and an ATP-dependent Cl^- pump (Chipperfield et al., 1993; Davis, 1992, 1996; Davis et al., 1993). Norepinephrine activates inward Cl^- transport and intracellular Cl^- actually increases in the presence of the agonist (Davis et al., 1997). This occurs despite the concomitant activation of Cl^- conductance and associated increase in Cl^- efflux. This agonist-induced enhancement of inward Cl^- transport may be critical for maintaining sustained Cl^- dependent depolarization.

* Corresponding author. Tel.: +1-319-356-3978; fax: +1-319-353-8957.
E-mail address: fred-lamb@uiowa.edu (F.S. Lamb).

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Two specific types of Cl^- conductance, a Ca^{2+}-dependent Cl^- current and a volume-activated Cl^- current, have been identified in both vascular smooth muscle and endothelial cells. The Ca^{2+}-activated Cl^- channel can be activated by Ca^{2+} influx or by Ca^{2+} release from intracellular stores (Large and Wang, 1996). The volume-activated Cl^- channel is activated in response to cell swelling (Nilius et al., 1994) or reduction in intracellular ionic strength (Nilius et al., 1998). This current is also regulated by protein tyrosine phosphorylation and G-proteins (Voets et al., 1998). In elastic tissues such as blood vessels, it is not known if volume-activated Cl^- current can be activated by mechanical stretch.

Considerable evidence for the functional importance of Cl^- channels in vascular smooth muscle has been obtained from isolated conduit (Criddle et al., 1996; Lamb and Barna, 1998a,b; Nelson et al., 1997) and mesenteric resistance vessels (Criddle et al., 1997). In order to determine if Cl^- currents contribute to the response of resistance vessels to norepinephrine in vivo, and to compare these effects in three different vascular beds, we examined the hemodynamic effects of 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS) in anesthetized rats. DIDS inhibits the Cl^- bicarbonate exchanger, which will lower intracellular Cl^- in vascular smooth muscle and, thereby, limit Cl^- current-mediated depolarization (Davis, 1992). DIDS also blocks Ca^{2+}-activated Cl^- channels (Hogg et al., 1994; Lamb et al., 1994) and volume-activated Cl^- channels (Greenwood and Large, 1998) in vascular smooth muscle which will prevent activation of these depolarizing currents. Importantly, DIDS does not directly inhibit voltage-dependent Ca^{2+} channels (Ca^{2+} vs. -channels) (Lamb and Barna, 1998a; Lamb et al., 1994). We hypothesized that interfering with Cl^- metabolism would suppress regional vasoconstrictor responses to norepinephrine in vivo. In this study, we determined: (1) the effect of DIDS, or the Ca^{2+} vs. -channel blocker, nifedipine, on resting mean arterial blood pressure and vascular resistances in pentobarbital-anesthetized rats, (2) the effect of DIDS or nifedipine on the hemodynamic actions of norepinephrine. The effects of DIDS and nifedipine were compared in order to provide evidence as to whether interfering with Cl^- channel-mediated depolarization would prevent the opening of Ca^{2+} vs. -channels in resistance arteries.

2. Materials and methods

2.1. Animals

The University of Iowa Institutional Animal Care and Use Committee approved the protocols described here. Male Sprague–Dawley rats (250–300 g) were used in these studies.

2.2. Catheterization and placement of Doppler flow probes

Each rat was anesthetized with pentobarbital (50 mg/kg, i.p.). A catheter was placed into a femoral artery to measure mean arterial blood pressure. A catheter was also placed in a femoral vein to give drugs. Pentobarbital (5 mg, i.v.) was given as needed to maintain anesthesia. A midline laparotomy was performed and miniature pulsed Doppler flow probes were placed on the superior mesenteric artery, renal artery and abdominal aorta (below the renal arteries), to record mesenteric, renal and hindquarter blood flows and to determine vascular resistances in these beds. Details of the Doppler technique, including construction of probes, reliability of the method for estimation of flow velocity, and quantitative determination of percentage changes in vascular resistance have been described previously (Haywood et al., 1981). The arterial catheter was connected to a Beckman Dynograph-coupled pressure transducer (Cobe Laboratories) to measure mean arterial pressure. The wire leads from the flow probes were connected to a Beckman Dynograph-coupled pulsed-Doppler flowmeter (Department of Bioengineering, University of Iowa). Data were recorded on a MacLab 8E and stored on a Power MacIntosh 7200 computer.

2.3. Experimental protocols

2.3.1. Dose-dependent hemodynamic effects of DIDS

The effects of DIDS (2.5–50 μmol/kg, i.v.) on mean arterial pressure and vascular resistances were examined. Hemodynamic parameters were recorded before and after administration of DIDS ($n = 5$) or an equal volume of saline (0.9% NaCl, i.v., $n = 5$). DIDS was given as bolus doses of 2.5, 5.0, 10, 25 and 50 μmol/kg for a total initial dose of 92.5 μmol/kg. Data were recorded again after administration of an additional 50 μmol/kg of DIDS (total dose of 142.5 μmol/kg, i.v.). Both the peak (initial maximum) and the sustained (following return of a stable baseline) effects of DIDS were recorded. The total volume required for the administration of DIDS was approximately 500 μl. The effects of each dose were allowed to subside completely (5–10 min), prior to the administration of subsequent doses.

2.3.2. The effect of DIDS on the hemodynamic actions of norepinephrine

The hemodynamic effects of norepinephrine (1.0, 2.5, 5.0 μg/kg, i.v.) were determined in the same animals described above. Data were recorded before and after the dose–response to DIDS (total dose of 92.5 μmol/kg), and again, 30–45 min after administration of the additional 50 μmol/kg of DIDS (total dose of 142.5 μmol/kg, i.v.). The first post-treatment injection of norepinephrine was given 10–15 min after the administration of the last dose

of DIDS when the hypotensive and vasodilator effects had subsided.

2.3.3. Hemodynamic effects of nifedipine

The effect of nifedipine (500 nmol/kg, i.v.) on mean arterial pressure and vascular resistances was examined. Nifedipine ($n = 6$) or an equal volume of vehicle (1% dimethylsulfoxide in 0.9% NaCl, $n = 6$) was given as a bolus dose in a total volume of approximately 250 μl. Both the peak (initial maximum) and the sustained (following return of a stable baseline) effects of nifedipine were recorded.

2.3.4. The effect of nifedipine on the hemodynamic actions of norepinephrine

The hemodynamic effects of norepinephrine (1.0, 2.5, 5.0 μg/kg, i.v.) were determined before and after exposure to equal volumes of either nifedipine (500 nmol/kg)

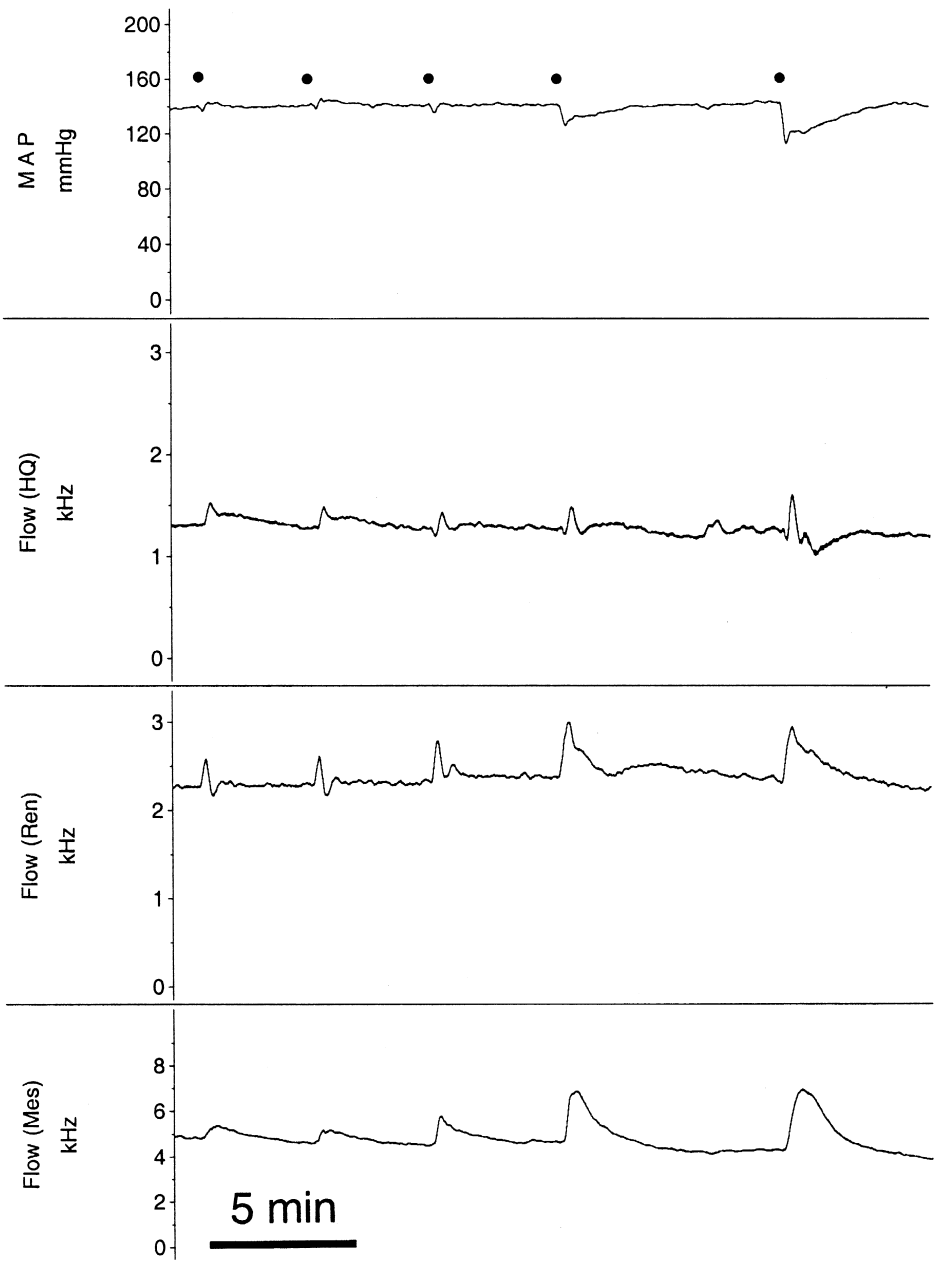

Fig. 1. Typical recording of a dose–response to DIDS (2.5, 5, 10, 25 and 50 μmol/kg, i.v.). The top tracing represents mean arterial pressure (MAP, mm Hg), the lower tracings represent hindquarter (Hind), renal (Ren) and mesenteric (Mes) blood flow (kHz). DIDS, administered at (●) in mean arterial pressure tracing, produced dose-dependent falls in mean arterial pressure, which were associated with increases in blood flow to all three beds. These changes are consistent with DIDS producing dose-dependent falls in hindquarter, renal and mesenteric resistance. In this example, the blood pressure lowering effect of DIDS lasted for 250 s.

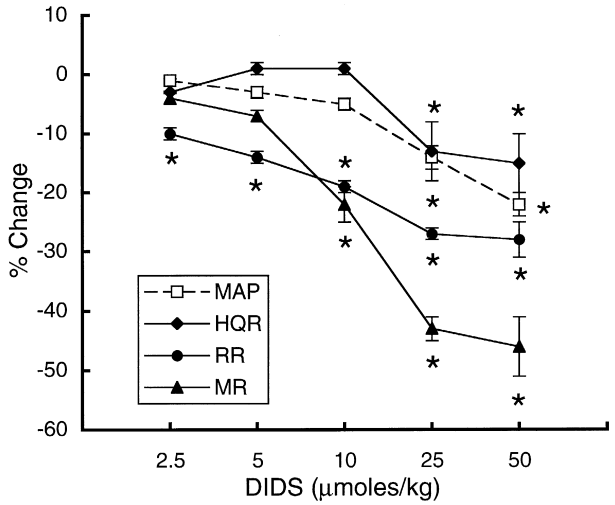

Fig. 2. Summary of the effects of DIDS (2.5–50 μmol/kg, i.v.) on mean arterial blood pressure (MAP) and hindquarter (HQR), renal (RR) and mesenteric (MR) vascular resistances of pentobarbital-anesthetized rats ($n = 5$). Each value represents the mean ± SE of the maximal percent changes in these parameters. * $P < 0.05$, significant change from pre-injection values.

or its vehicle. The first post-treatment injection of norepinephrine was given after the effects of nifedipine had stabilized (30–45 min).

2.4. Drugs

DIDS, nifedipine and norepinephrine were obtained from Sigma (St. Louis, MO, USA). DIDS was dissolved in saline. Nifedipine was dissolved in 1% DMSO in 0.9% NaCl. Sodium pentobarbital and sterile saline were obtained from Abbott Laboratories (Chicago, IL, USA).

2.5. Statistics

Data are expressed as the mean ± SE of the actual hemodynamic parameters and percent changes in these parameters. The area under the curve for the changes in mean arterial pressure (mm Hg × s) were also determined. The data were analyzed by repeated measures analysis of variance (ANOVA) (Winer, 1971), followed by Student's modified *t*-test with the Bonferroni correction for multiple comparisons between means using the modified error mean square term from the ANOVA (Wallenstein et al., 1980). A value of $P < 0.05$ was taken to denote statistical significance.

3. Results

3.1. Hemodynamic responses produced by systemic injections of DIDS

A typical example of the effects of successive injections of DIDS (2.5–50 μmol/kg, i.v.) on mean arterial pressure and hindquarter, renal and mesenteric blood flows in a naive rat is shown in Fig. 1. DIDS produced dose-dependent falls in mean arterial pressure, which were associated with relatively minor increases in blood flows. These changes are consistent with DIDS producing dose-dependent falls in hindquarter, renal and mesenteric resistances (i.e., vasodilator responses). The blood pressure lowering effect of the highest dose of DIDS lasted for 242 ± 5.7 s.

A summary of the hemodynamic effects of DIDS is shown in Fig. 2. DIDS produced dose-dependent falls in mean arterial pressure and in hindquarter, renal and mesenteric resistances. The vasodilator action of DIDS was most

Table 1
Summary of the hemodynamic values recorded over the times at which the effects of norepinephrine were determined in rats treated with saline or DIDS

Parameter	Treatment	Pre	Post Injection		% Changes	
			Post I	Post II	Post I vs. pre	Post II vs. pre
MAP, mm Hg	Saline	112 ± 4	113 ± 3	109 ± 3	+1 ± 2	−2 ± 2
	DIDS	117 ± 4	122 ± 6	126 ± 5	+4 ± 1*	+7 ± 1*
HQR, mm Hg/kHz	Saline	74 ± 9	68 ± 11	67 ± 12	−7 ± 5	−9 ± 6
	DIDS	98 ± 2	120 ± 8	137 ± 11	+23 ± 7*	+40 ± 13*
RR, mm Hg/kHz	Saline	83 ± 14	78 ± 12	86 ± 12	−6 ± 4	+3 ± 6
	DIDS	58 ± 7	57 ± 5	71 ± 7	0 ± 7	+26 ± 10*
MR, mm Hg/kHz	Saline	33 ± 5	37 ± 6	31 ± 5	+12 ± 8	−6 ± 6
	DIDS	26 ± 2	35 ± 2	40 ± 2	+41 ± 12*	+60 ± 14*

Each value represents the mean ± SE of actual values and the percent changes in these values. MAP = mean arterial blood pressure. HQR = hindquarter vascular resistance. RR = renal vascular resistance. MR = mesenteric vascular resistance. Five saline-treated rats received five intravenous doses of saline (Post I), followed by a sixth intravenous doses of saline (Post II). Five other rats received an initial total cumulative intravenous dose of 92.5 μmol/kg of DIDS (total of 5 injections, Post I), and then, an additional 50 μmol/kg dose of this Cl^- channel blocker (Post II, total dose of 142.5 μmol/kg, i.v.). In these rats, injections of norepinephrine were given between 10 and 25 min after the first set of injections of saline or DIDS, and again, between 10 and 25 min after the final injection of saline or DIDS.

* $P < 0.05$, significant change from pre values.

prominent in the mesenteric bed, less evident but still pronounced in the renal bed, and quite minimal in the hindquarter bed. The hemodynamic values recorded after recovery from the hypotensive and vasodilator effects of the initial cumulative dose of DIDS (92.5 μmol/kg) and after a second dose of DIDS (50 μmol/kg, total dose of 142.5 μmol/kg) are summarized in Table 1. The hemodynamic values recorded before and after two control injections of saline are also shown. The injections of saline did not elicit immediate or delayed hemodynamic responses ($P > 0.05$ for all responses, data not shown). The post-injection values are those recorded during the time the hemodynamic effects of norepinephrine were examined (see below). Mean arterial pressure, hindquarter and mesenteric resistance were higher than pre-injection values after recovery from the hypotensive and vasodilator actions of the initial 92.5 μmol/kg dose of DIDS ($P < 0.05$ for all comparisons). Mean arterial pressure, hindquarter, renal and mesenteric resistances were all significantly higher than pre-injection values after recovery from the initial effects of the second dose of DIDS ($P < 0.05$ for all comparisons).

3.2. Effect of DIDS on the maximal responses produced by norepinephrine

A typical example of the effects of norepinephrine (5 μg/kg, i.v.) on mean arterial pressure and hindquarter, renal and mesenteric blood flows before and after administration of DIDS (142.5 μmol/kg, i.v.) is shown in Fig. 3. Prior to the administration of DIDS, this dose of norepinephrine produced a pronounced increase in mean arterial pressure lasting approximately 120 s. The increase in mean arterial pressure was associated with an increase in hindquarter blood flow and marked falls in renal and mesenteric blood flows. These changes are consistent with this dose of norepinephrine, producing no significant change in hindquarter resistance and pronounced increases in renal and mesenteric resistances. After administration of DIDS, norepinephrine produced similar initial increases in mean arterial pressure, hindquarter and renal resistance. However, the duration (approximately 75 s) of the norepinephrine-induced rise in mean arterial pressure was markedly diminished after administration of DIDS.

Fig. 4 shows the peak hemodynamic responses produced by norepinephrine (1–5 μg/kg, i.v.) before and after cumulative administration of DIDS (2.5–50 μmol/kg, i.v., 92.5 μmol/kg total dose), and again, after a subsequent 50 μmol/kg dose of DIDS (total dose of 142.5 μmol/kg). Norepinephrine produced dose-dependent increases in mean arterial pressure, renal resistance and mesenteric resistance. In contrast, the lower doses of norepinephrine produced vasodilatation in the hindquarter bed. The peak pressor responses produced by the lower doses of norepinephrine were somewhat diminished after the administration of the first dose of DIDS. DIDS had no effect on the peak response to 5.0 μmol/kg norepinephrine. The second dose of DIDS did not cause significant further suppression of the peak norepinephrine-induced pressor responses. The maximal vasoconstrictor effects of

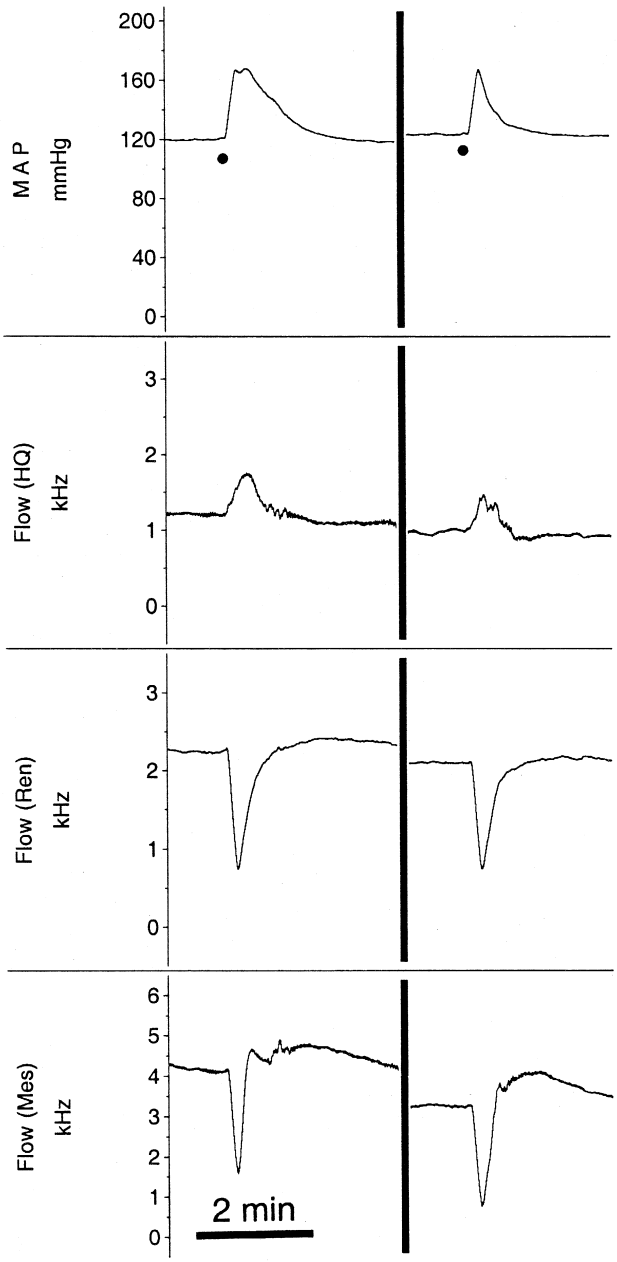

Fig. 3. Typical example of changes in mean arterial blood pressure (BP) and blood flow (kHz) in hindquarter (Ao), renal (Ren) and mesenteric (Mes) vascular beds elicited by injection of norepinephrine (5 μg/kg, i.v.) before (left of solid vertical bar) and after (right of solid vertical bar) administration of DIDS (142.5 μmol/kg). The injections were separated by 45 min. Before injection of DIDS, norepinephrine elicited an increase in MAP of about 120 s. The increase in MAP was associated with a small increase in hindquarter blood flow but marked falls in renal and mesenteric blood flows. As such, norepinephrine produced no change in hindquarter but pronounced increases in renal and mesenteric resistances. After administration of DIDS, norepinephrine produced similar initial increases in MAP and vascular resistances. However, the durations (75 s) of the norepinephrine-induced responses were markedly diminished.

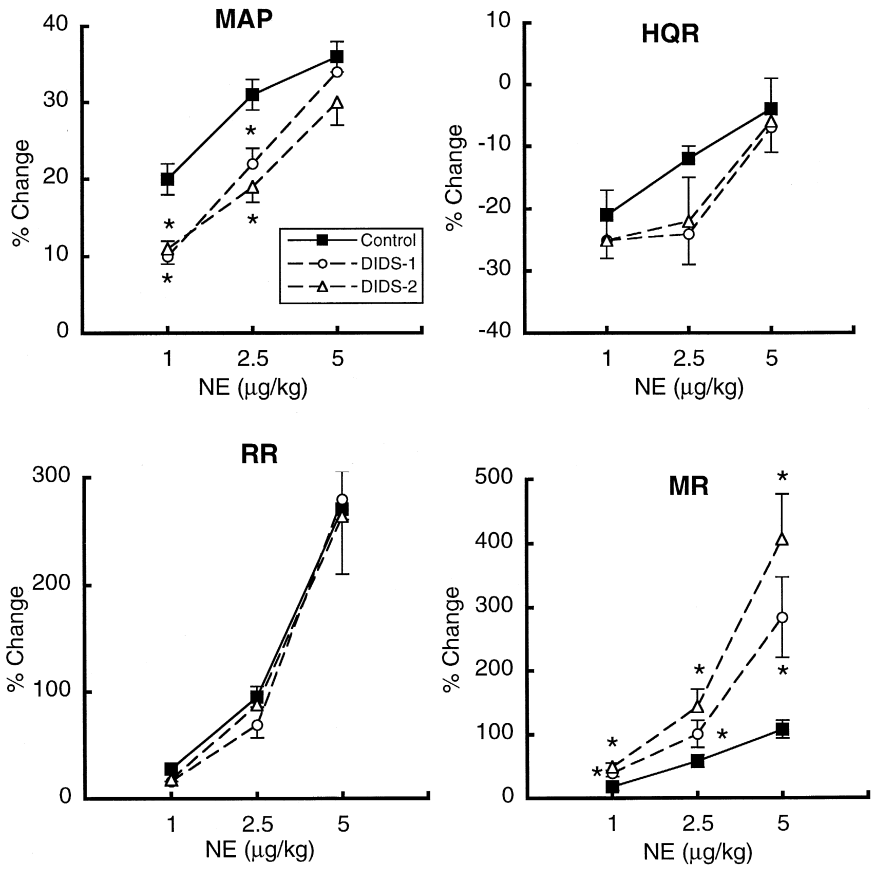

Fig. 4. Summary of effects of norepinephrine (1–5 μg/kg, i.v.) on mean arterial blood pressure (MAP) and hindquarter (HQR), renal (RR) and mesenteric (MR) vascular resistances of pentobarbital-anesthetized rats ($n = 5$) before and after intravenous administration of a 92.5 μmol/kg dose of DIDS (DIDS-1) and then a 50 μmol/kg dose of DIDS (DIDS-2, total dose of 142.5 μmol/kg, i.v.). Each value represents the mean ± SE of the maximal percent changes in these parameters. * $P < 0.05$, significant difference from control responses.

norepinephrine in the renal bed and the vasodilator effects in the hindquarter bed were unaffected by DIDS. In contrast, the maximal vasoconstrictor effects of norepinephrine in the mesenteric bed were markedly augmented after the administration of DIDS. The hemodynamic effects of these doses of norepinephrine were similar before and after administration of saline ($P > 0.05$ for all comparisons, data not shown).

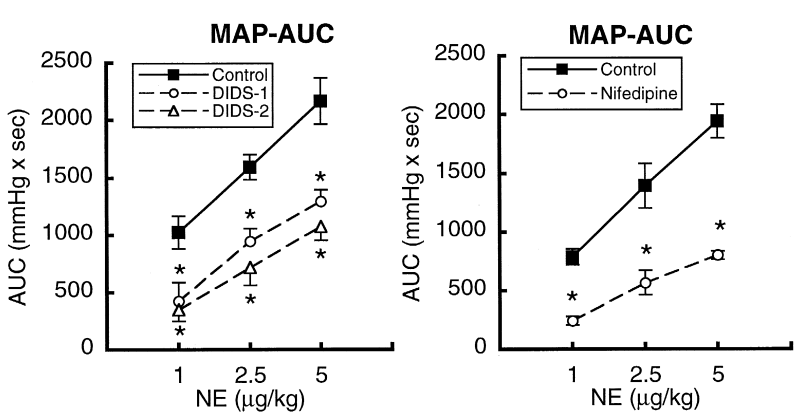

Fig. 5. Left panel: summary of total increases in mean arterial blood pressure (MAP) expressed as area under the curve (AUC, mm Hg × s) elicited by norepinephrine (1–5 μg/kg, i.v.) in pentobarbital-anesthetized rats ($n = 5$) before and after intravenous injection of 92.5 μmol/kg of DIDS (DIDS-1) and then 50 μmol/kg of DIDS (DIDS-2, total dose, 142.5 μmol/kg, i.v.). Right panel: summary of total increases in MAP elicited by norepinephrine (1–5 μg/kg, i.v.) in pentobarbital-anesthetized rats ($n = 6$) before and after administration of nifedipine (500 nmol/kg, i.v.). Each value represents the mean ± SE of the maximal percent changes in these parameters. * $P < 0.05$, significant difference from control values.

Table 2
Summary of the hemodynamic values recorded before and after the injection of saline or nifedipine

Parameter	Treatment	Pre	Post Injection		% Changes	
			Maximum	Recovery	Maximum vs. pre	Recovery vs. pre
MAP, mm Hg	Vehicle	112 ± 3	110 ± 3	109 ± 3	−2 ± 2	−3 ± 2
	Nifedipine	115 ± 3	70 ± 5	112 ± 4	−38 ± 7*	−2 ± 2
HQR, mm Hg/kHz	Vehicle	88 ± 14	86 ± 13	85 ± 12	−2 ± 4	−3 ± 3
	Nifedipine	76 ± 7	35 ± 6	73 ± 8	−53 ± 9*	−3 ± 4
RR, mm Hg/kHz	Vehicle	63 ± 9	66 ± 10	57 ± 11	+5 ± 7	−11 ± 7
	Nifedipine	46 ± 8	28 ± 6	49 ± 5	−39 ± 5*	+7 ± 5
MR, mm Hg/kHz	Vehicle	25 ± 5	26 ± 5	22 ± 6	4 ± 7	−11 ± 9
	Nifedipine	32 ± 4	22 ± 5	39 ± 4	−31 ± 7*	+21 ± 6*

Each value represents the mean ± SE of actual values and the percent changes in these values. MAP = mean arterial blood pressure. HQR = hindquarter vascular resistance. RR = renal vascular resistance. MR = mesenteric vascular resistance. Six rats received an intravenous dose of vehicle and six rats received an intravenous dose of nifedipine (500 nmol/kg). The recovery period is the time over which the rats received intravenous injections of norepinephrine. In these rats, injections of norepinephrine were given between 30–45 min after the injection of vehicle or nifedipine.

* $P < 0.05$, significant change from pre values.

3.3. Effect of DIDS on the total increases in mean arterial pressure produced by norepinephrine

A summary of the total increases in mean arterial pressure elicited by norepinephrine (1–5 μg/kg, i.v.) before and after cumulative administration of 92.5 μmol/kg of DIDS, and again, after administration of 50 μmol/kg of DIDS (total dose of 142.5 μmol/kg, i.v.) is shown in the left hand panel of Fig. 5. The total pressor response is expressed as area under the curve (mm Hg × s) for the increase in mean arterial pressure over time. The total norepinephrine-induced pressor responses increased with dose. The total pressor responses were markedly reduced after administration of 92.5 μmol/kg of DIDS, but were not further depressed after administration of an additional 50 μmol/kg dose of DIDS (total dose of 142.5 μmol/kg, i.v.). The total pressor responses produced by these doses of norepinephrine were similar before and after two injec-

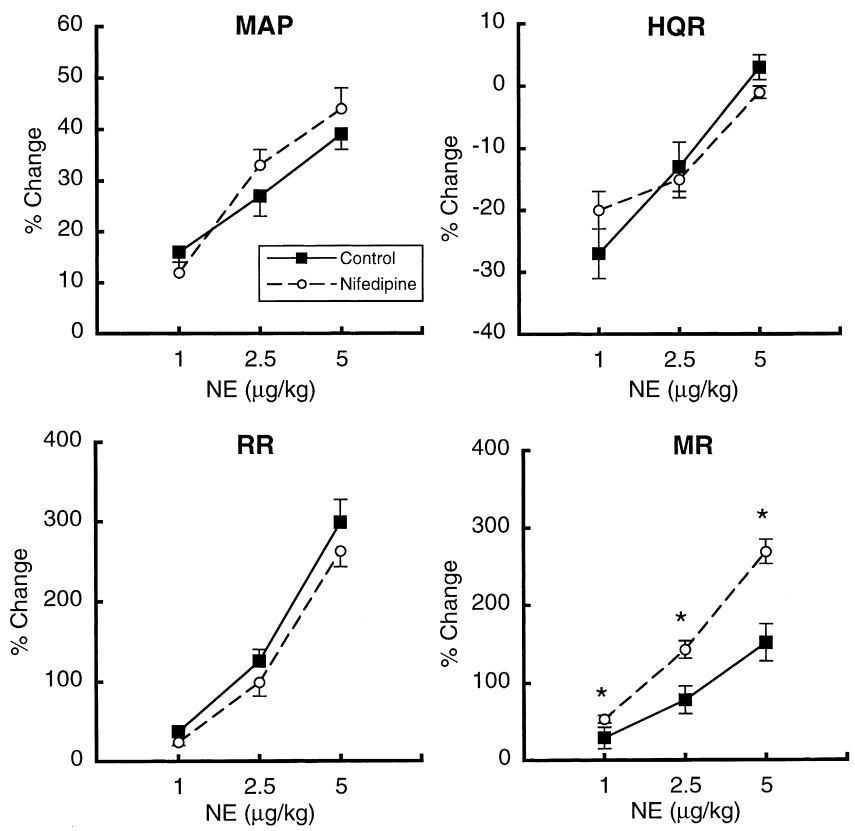

Fig. 6. Summary of the effects of norepinephrine (1–5 μg/kg, i.v.) on mean arterial blood pressure (MAP) and hindquarter (HQR), renal (RR) and mesenteric (MR) vascular resistances of pentobarbital-anesthetized rats ($n = 6$) before and after administration of nifedipine (500 nmol/kg, i.v.). Each value represents the mean ± SE of the maximal percent changes in these parameters. * $P < 0.05$, significant difference from control values.

tions of saline ($P > 0.05$ for all comparisons, data not shown).

3.4. Hemodynamic response produced by systemic injection of nifedipine

A summary of the effects of nifedipine (500 nmol/kg, i.v.) on resting hemodynamic variables is shown in Table 2. Nifedipine produced rapid falls in mean arterial pressure and vascular resistances. Mean arterial pressure, hindquarter and renal resistance returned to pre-injection values after 20–30 min. However, mesenteric resistance returned to values slightly above pre-injection values ($P < 0.05$). This increase in mesenteric resistance lasted for about 30–45 min. Injection of the vehicle used to dissolve nifedipine did not affect resting hemodynamic variables.

3.5. Effect of nifedipine on the maximal responses produced by norepinephrine

A summary of the effects of norepinephrine (1–5 μg/kg, i.v.) on mean arterial pressure and hindquarter, renal and mesenteric resistances of pentobarbital-anesthetized rats before and after administration of nifedipine (500 nmol/kg, i.v.) is shown in Fig. 6. Control responses to norepinephrine produced changes in mean arterial pressure and vascular resistances that were very similar to those measured when the same doses were given to the group of animals which subsequently received DIDS. The maximal pressor responses produced by norepinephrine were unaffected by nifedipine ($P > 0.05$ for all comparisons). In addition, nifedipine did not alter the maximal norepinephrine-induced changes in hindquarter or renal resistance ($P > 0.05$ for all comparisons). However, as with DIDS, the vasoconstrictor responses produced by each dose of norepinephrine in the mesenteric bed were augmented after administration of nifedipine ($P < 0.05$ for all comparisons). The hemodynamic responses to these doses of norepinephrine were similar before and after administration of vehicle ($P > 0.05$ for all comparisons, data not shown).

3.6. Effect of nifedipine on total increases in mean arterial pressure produced by norepinephrine

A summary of the total increases in mean arterial pressure produced by norepinephrine (1–5 μg/kg, i.v.) before and after administration of nifedipine (500 nmol/kg, i.v.) is shown in the right hand panel of Fig. 5. The total pressor responses (mm Hg × s) produced by each dose of norepinephrine were markedly diminished after administration of nifedipine ($P < 0.05$ for all comparisons). The total pressor responses produced by these doses of norepinephrine were similar before and after injection of vehicle ($P > 0.05$ for all comparisons, data not shown).

4. Discussion

4.1. Initial vasodilator effects of DIDS and nifedipine

DIDS blocks Cl^- channels and inhibits Cl^- bicarbonate exchange (Davis, 1992; Greenwood and Large, 1998; Lamb et al., 1994). Systemic injections of DIDS produced dose-dependent reductions in mean arterial pressure. These depressor responses were associated with vasodilator responses that were most pronounced in the mesenteric bed, weaker in the renal bed, and minimal in the hindquarter beds. The prominent vasodilation produced by DIDS in anesthetized rats is consistent with the pronounced relaxation produced in isolated rat aorta (Lamb and Barna, 1998a) and cerebral blood vessels (Nelson et al., 1997). The in vitro vasodilator effect of DIDS does not require an intact endothelium (Lamb and Barna, 1998b). Therefore, the degree to which DIDS would produce vasodilation in a vascular bed should be a function of the contribution of DIDS-sensitive Cl^- conductance to membrane potential of arteriolar vascular smooth muscle in that bed.

The DIDS responses lasted from 30 s to 4 min, depending on dose. Higher doses of DIDS may have produced larger falls in mean arterial pressure and vascular resistance. However, the responses elicited by 50 μmol/kg dose of DIDS were not markedly different to those elicited by a 25 μmol/kg dose. Recovery of mean arterial pressure following DIDS is likely to involve active compensatory processes rather than simple clearance of DIDS from the circulation, as DIDS-induced alterations in response to norepinephrine persisted long after arterial pressure had recovered (see below). The vasodilator effects of DIDS in the hindquarter and renal beds were considerably smaller than those of nifedipine. In contrast, the DIDS-induced fall in mesenteric resistance was equal to that of nifedipine. Since increasing Cl^- conductance is only one mechanism that produces depolarization and activation of Ca^{2+} vs. -channels, it is not surprising that DIDS was a less potent vasodilator than nifedipine in some beds. The more pronounced effect of DIDS in the mesenteric bed may indicate that Cl^- conductance is of particular importance in this bed. This idea is supported by the data from isolated, perfused rat mesenteric beds, showing that a different Cl^- channel blocker niflumic acid was as effective as nifedipine at blocking vasoconstriction to norepinephrine and the two drugs together were no more effective than niflumic acid alone (Criddle et al., 1997).

Other compounds that interfere with Cl^- transport are known to be vasoactive. Loop diuretics which inhibit Na^+–K^+–$2Cl^-$ co-transport, such as furosemide and bumetanide, are vasodilator agents in vitro (Barthelmebs et al., 1994; Greenberg et al., 1994; Lamb and Barna, 1998a) and in vivo (Dikshit et al., 1973; Kelly, 1994). They are particularly effective at eliciting venodilatation (Greenberg et al., 1994). This may explain the ability of these diuretics

to produce relief from symptoms of congestive heart failure in the absence of significant diuresis. The effect of these compounds on arterial smooth muscle is less pronounced (Greenberg et al., 1994), which explains their lack of immediate effect upon blood pressure. Both Na^+–K^+–$2Cl^-$ co-transport and Cl^- bicarbonate exchange are important for maintaining normal intracellular Cl^- concentration in arterial smooth muscle (Davis, 1992). The depressor effects of DIDS may be more pronounced than those of the loop diuretics because DIDS blocks Ca^{2+}-activated (Lamb et al., 1994) and swelling-activated (Strange et al., 1996) Cl^- channels, as well as Cl^- bicarbonate exchange. The effects of DIDS in vivo are unlikely to result from direct inhibition of L-type Ca^{2+} vs. -channels since DIDS does not inhibit these channels in isolated cells (Lamb et al., 1994) and does not alter K^+-induced (Ca^{2+} vs. -channel-dependent) contraction in isolated arteries (Lamb and Barna, 1998a).

4.2. Delayed effects of DIDS and nifedipine on arterial pressure and vascular resistances

After recovery from the vasodilator effects of DIDS, mean arterial pressure and vascular resistances returned to values that were slightly higher than pre-injection. After recovery from nifedipine-induced vasodilatation, mean arterial pressure returned to pre-injection levels, and hindquarter and renal resistance were unchanged. Both DIDS and nifedipine produced a sustained rise in mesenteric resistance. The mechanism of the delayed vasoconstriction in response to DIDS may be related to altered endothelial cell Cl^- metabolism (Hosoki and Iijima, 1994; Song and Davis, 1994). In isolated endothelial cells, altering Cl^- gradient prevents agonist-induced sustained rises in intracellular Ca^{2+}, which are linked to production and release of endothelium-derived relaxing factors (EDRFs) (Luckhoff et al., 1988). Nifedipine should not affect endothelial function since these cells lack dihydropyridine-sensitive Ca^{2+} vs. -channels. The mechanism by which nifedipine caused a sustained increase in mesenteric resistance is unknown.

4.3. Effect of DIDS and nifedipine on hemodynamic actions of norepinephrine

Norepinephrine elicited dose-dependent increases in mean arterial pressure which were associated with vasoconstrictor responses in renal and mesenteric beds and vasodilatation in the hindquarter bed. The hindlimb vasodilatation reflects the high density of β-adrenoceptors present in this bed (Kooy and Lewis, 1996). The vasoconstrictor effects of norepinephrine are primarily due to stimulation of α_1-adrenoceptors on vascular smooth muscle. Activation of phospholipase C leads to inositol phosphate-mediated release of intracellular Ca^{2+} stores and diacylglycerol-mediated activation of protein kinase C. The early vasoconstrictor effects of norepinephrine in anesthetized rats are due largely to the activation of contractile proteins by Ca^{2+} from sarcoplasmic reticulum (Abdel-Latif, 1986). Subsequent vasoconstriction requires entry of extracellular Ca^{2+}, largely through Ca^{2+} vs. -channels (Abdel-Latif, 1986). A component of the depolarization required to activate Ca^{2+} vs. -channels is dependent upon Ca^{2+}-activated Cl^- channels, which are triggered by release of intracellular Ca^{2+} stores (Lamb et al., 1994; Large and Wang, 1996; Pacaud et al., 1989). Inhibition of this Cl^- current by DIDS (Lamb et al., 1994) should block depolarization and therefore prevent extracellular Ca^{2+} entry. The peak response to norepinephrine was somewhat diminished by DIDS at low norepinephrine doses (1.0, 2.5 but not 5.0 $\mu g/kg$), while these responses were unaffected by nifedipine. The mechanism of this effect of DIDS is unclear but is likely to be unrelated to its ability to block Cl channels and inhibit voltage-dependent Ca^{2+} entry. In contrast, the total vasoconstrictor effect (mm Hg × s) of norepinephrine was similarly attenuated by both compounds. This suggests that following DIDS treatment, despite the presence of normally functioning Ca^{2+} vs. -channels, a poorly sustained vasoconstriction may be produced due to a lack of Cl^- channel-dependent depolarization.

It is curious that peak vasoconstrictor responses to norepinephrine are actually increased by both DIDS and nifedipine in the mesenteric bed. Norepinephrine may be able to mobilize intracellular Ca^{2+} stores more effectively under these conditions. Resting resistance in the mesenteric bed was elevated after recovery from the vasodilator responses elicited by nifedipine ($+21 \pm 6\%$, Table 2), whereas resting resistances were higher after recovery from the vasodilator responses of DIDS in each vascular bed. However, the rise in resistance post-DIDS was highest in the mesenteric bed ($+60 \pm 14\%$, Table 1). It is possible that the increased peak response to norepinephrine in the mesenteric bed is related to this increase in resting tone.

4.4. Summary

This study presents evidence that there is a Cl^- dependence to α-adrenoceptor-mediated contraction of resistance vessels in the rat. The degree to which DIDS dilates arterioles in a vascular bed may be related to the mechanisms regulating resting tone. Vessels with a large degree of α-adrenergic tone may be more susceptible to disruption of Cl^- metabolism and dilate more prominently. Accordingly, Cl^- channel blockers may constitute an interesting new class of anti-hypertensive agents.

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