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1-Ethyl-2-benzimidazolinone stimulates endothelial K_{Ca} channels and nitric oxide formation in rat mesenteric vessels

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Abstract

Hyperpolarization of most blood vessels occurs by the opening of K_{Ca} channels. 1-Ethyl-2-benzimidazolinone (1-EBIO) is a direct activator of K_{Ca} channels in epithelial cells and is potentially valuable for studying cellular hyperpolarization. This study reports the effects of 1-EBIO on isolated rat mesenteric beds perfused with normal (4.7 mM), or high (20 or 80 mM) K^+ physiological salt solution (PSS) and constricted with an α_1-adrenoceptor agonist, cirazoline (0.3–1 μM). Arterial perfusion pressures were decreased by 1-EBIO (0.1–30 nmol) in a dose- and endothelium-dependent manner. Infusion of penitrem A (100 nM), a maxi-K^+ channel blocker, or apamin (0.5 μM), a small-conductance (SK_{Ca}) K^+ channel blocker, produced significant increases in cirazoline-mediated tone (mm Hg): 103.3 ± 8.7 (control) vs. 156.3 ± 14.3 (penitrem A); or 93.0 ± 15.8 (control) vs. 114.0 ± 15.4 (apamin). 1-EBIO relaxations were attenuated by penitrem A, while apamin, dendrotoxin (50 nM; a K_V channel antagonist), or ouabain (100 μM; a sodium pump blocker) failed to alter the responses. 1-EBIO-mediated relaxations decreased significantly with increasing extracellular [K^+]: relaxations to 30 nmol were $89.3\% \pm 3.2\%$ (4.7 mM K^+, normal PSS) vs. $59.5\% \pm 3.4\%$ and $19.0\% \pm 3.9\%$ for 20 and 80 mM K^+ PSS, respectively. *N*ω-nitro-L-arginine-methyl ester (L-NAME; 100 μM), and 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one (ODQ; 10 μM), selective inhibitors of nitric oxide synthase, and nitric oxide-sensitive guanylate cyclase, respectively, abolished 1-EBIO relaxations in vessels perfused with 20 or 80 mM K^+ PSS. We conclude that: (1) maxi-K^+ and SK_{Ca} channels are present in rat mesenteric arterial vessels and actively contribute to vascular tone, (2) vasodilator action of 1-EBIO involves the opening of endothelial maxi-K^+ channels and nitric oxide synthesis.

Keywords: Mesenteric vessel; 1-Ethyl-benzimidazolinone; K_{Ca} channel opener

1. Introduction

Potassium (K^+) channels constitute a diverse and large group of transmembrane channels serving very different functions (Edwards and Weston, 1990; Kuriyama et al., 1995). Changes in activity of these channels represent a major mechanism that regulates vascular tone. Activation of K^+ channels in vascular smooth muscle hyperpolarizes cell membranes and closes voltage-dependent calcium channels. These actions decrease intracellular calcium and cause vascular smooth muscle relaxation (Nelson, 1993; Nelson and Quayle, 1995). Four major types of K^+ channels appear to be involved with vascular regulation. They include: (1) ATP-sensitive (K_{ATP}), (2) Ca^{2+}-dependent (K_{Ca}), (3) voltage-dependent or delayed rectifier (K_V), and (4) inward-rectifier potassium channels. Glibenclamide, iberiotoxin/tetraethylammonium, and 4-aminopyridine/dendrotoxin fairly selectively inhibit K_{ATP}, K_{Ca}, and K_V channels, respectively, while low concentrations of extra-cellular barium ions inhibit inward rectifier potassium channels (Faraci and Heistad, 1998).

Activators of K^+ channels are numerous and of diverse chemical classification. For example, pinacidil, cromakalim and diazoxide belong to thiourea/cyanoguanidine, benzopyran, and benzothiadiazine chemical groups, respectively. Because K^+ channel activity initiated by these compounds is blocked by physiological levels of ATP and by the antidiabetic sulfonylurea, glibenclamide (Quast and Cook, 1989; Standen et al., 1989), they are

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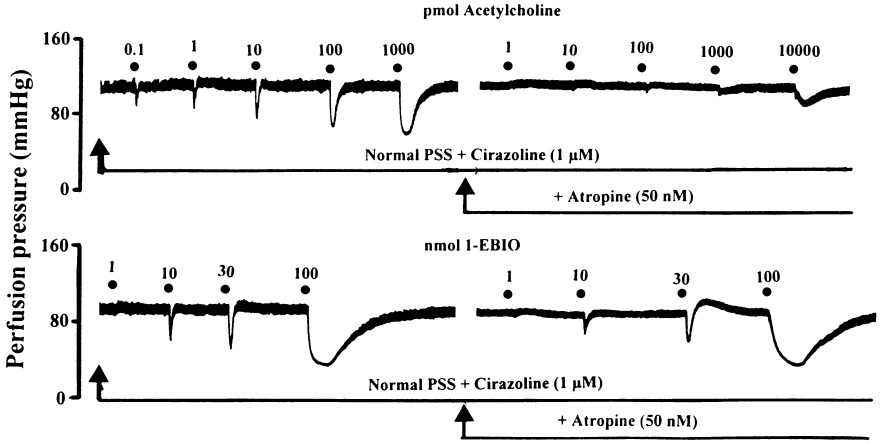


Fig. 1. Differential effects of atropine (50 nM) on dilator responses initiated by acetylcholine (top panel) and 1-EBIO (lower panel) in isolated, perfused rat mesenteric vascular bed. Arterial tone was increased with cirazoline (1 μM) added to the perfusion medium.

classified as K_{ATP} activators or openers. However, a few studies also document that cromakalim opens the maxi-(Ca^{2+}-dependent-) K^+ channels in cultured cells of bovine and rabbit aorta (Kreye et al., 1987; Gelband et al., 1990).

Selective pharmacological activators of K_{Ca}, unlike K_{ATP} channels, are not common. Two generations of compounds have been introduced as selective K_{Ca} activators. Compound SCA40, 6-bromo-8-methylaminoimidazolo[1,2-*a*]pyrazine-2-carbonitrile (Bonnet et al., 1992), initiated smooth muscle relaxations that were blocked by charybdotoxin, and caused hypotension in vivo that was not modified by atropine, glyburide or popranolol (Laurent et al., 1993). The benzimidazolones (e.g., compounds NS 1619 and 1-EBIO), are a novel class of compounds characterized as direct activators of maxi-K^+ channels (Olesen et al., 1994; Sellers and Ashford, 1994; Devor et al., 1996a,b). Compound 1-EBIO activates Ca^{2+}-dependent K^+ channels in the basolateral membrane of T84 epithelial cells resulting in a sustained Cl^- secretory response. These responses to 1-EBIO were blocked by charybdotoxin (Devor et al., 1996a,b). 1-EBIO has also recently been shown to be a selective opener of intermediate-conductance (IK_{Ca}) K^+ channels in cultured bovine aortic endothelial cells (Edwards et al., 1998).

Hyperpolarization of many blood vessels occurs by the opening of K_{Ca} channels which can be expressed in the endothelium (Fichtner et al., 1987; Luckhoff and Busse, 1990a,b; Sakai, 1990; Nilius, 1991; Vaca et al., 1992; Marchenko and Sage, 1996) as well as in smooth muscle cells (Kuriyama et al., 1995). We hypothesize that K_{Ca} channels contribute to the regulation of vascular tone. Therefore, objectives of our study were two-fold. First, to characterize with selective antagonists, the K^+ channel type(s) present in the rat mesenteric vasculature, and the contribution of such channels to the maintenance of mesenteric tone. Our preliminary studies show that compound 1-EBIO dilates rat mesenteric vascular beds perfused with physiological salt solution (PSS). Thus, the second objective of this study is to determine the role of endothelial vs. smooth muscle K^+ channels in 1-EBIO-mediated vasodilation. Selective K^+ channel antagonists used include: dendrotoxin (K_V), penitrem A (a maxi-K^+ or large-conductance Ca^{2+}-activated K^+ channel blocker), and apamin (a SK_{Ca} or small-conductance Ca^{2+}-activated K^+ channel blocker).

An abstract of the manuscript has been presented at the William Harvey Research Conferences on Endothelium: Gateway to New Therapeutics, Boston, MA (Adeagbo, 1999).

2. Methods

2.1. Perfused mesenteric vascular bed

Male Sprague–Dawley rats (250–350 g) were used in these studies. Following pentobarbital (60 mg/kg, i.p) anesthesia, the abdominal cavity of individual rats was opened and the superior mesenteric artery was cannulated through an incision at its confluence with the dorsal aorta. The entire mesenteric vascular bed was then flushed with heparinized PSS and the small intestinal borders were

Table 1
Influence of K^+ channel antagonists, or ODQ on vascular tone maintained with an infusion of 1 μM cirazoline. Data represent the means ± S.E.M., *n* in parenthesis

Treatment	% Change in perfusion pressure	
	Endo-intact	Endo-denuded
Apamin (0.5 μM)	25.0 ± 4.8 ($n = 5$)[a]	19.1 ± 6.7 ($n = 4$)[a]
Penitrem A (100 nM)	52.1 ± 9.7 ($n = 5$)[a]	46.1 ± 7.0 ($n = 4$)[a]
Dendrotoxin (50 nM)	0.9 ± 0.3 ($n = 4$)	Not done
ODQ (10 μM)	56.0 ± 7.5 ($n = 4$)[a]	7.9 ± 3.5 ($n = 4$)[b]

[a] $P < 0.05$ increases above cirazoline-induced tone.
[b] $P < 0.05$ from corresponding endo-intact value.

trimmed off. It was then transferred to a warmed (37°C) chamber where it was perfused with carbogen (95% O_2/5% CO_2)-saturated PSS maintained at 37°C. The perfusion was at a constant rate of 5 ml/min using a Masterplex peristaltic pump. Changes in perfusion pressure were recorded with Statham pressure transducers coupled to a Grass polygraph recorder (model 7H).

The composition of our PSS in mM was: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 12.5, Glucose 11.1. High K^+ (20 or 80 mM) PSS was prepared by substituting an equimolar amount of K^+ for Na^+ ions. The pH of all PSS was 7.4 after saturation with 95% O_2/5% CO_2 gas mixture. Chlorpheniramine and popranolol (0.1 μM) were added to the PSS to block the possible vasodilator effects of histamine and norepinephrine on histamine H_1 and β-adrenoceptors, respectively, following their release by high K^+ from mast cells and from sympathetic nerve terminals. Tissues were routinely allowed to equilibrate for 1 h before the start of all experiments. To establish whether compound 1-EBIO produced its effects through muscarinic cholinoceptors stimulation, we tested atropine (50 nM) for possible antagonizing effects on dilator responses to the compound.

2.2. Experimental protocol

2.2.1. Series 1

The first series was conducted to determine the effects of K^+ channel antagonists penitrem A (maxi-K^+), apamin (SK_{Ca}) or dendrotoxin (K_V) on perfusion pressure and on dilator responses initiated by 1-EBIO. To ensure adequate baseline vascular tone, the perfusion pressure of the vascular beds was increased by a continuous infusion of an α_1-selective adrenoceptor agonist cirazoline (1 μM). Dose–response curves to 1-EBIO were constructed in the absence, and in the presence of each antagonist, or a combination of any two of them. Antagonists were usually added to the perfusion medium 30 min prior to re-establishing 1-EBIO dose–response curve. Only one concentration of each antagonist (selected on the basis of available information in the literature), was employed in our study: penitrem A (100 nM), apamin (0.5 μM) and dendrotoxin (50 nM). We also tested the influence of a sodium pump blocker, ouabain (100 μM), on the dilator responses initiated by 1-EBIO.

2.2.2. Series 2

In the second series of experiments, we investigated the influence of extracellular potassium concentration on vasodilation initiated by 1-EBIO or acetylcholine. This was achieved by comparing dose–response curves to 1-EBIO or acetylcholine in vascular beds perfused with PSS containing 4.7 (normal), 20 and 80 mM K^+. Effects of *N*ω-nitro-L-arginine-methyl ester (L-NAME; 100 μM), and ODQ (10 μM), selective blockers of nitric oxide synthase, and nitric oxide-sensitive guanylate cyclase, respectively, were tested on the dilator responses to 1-EBIO and acetylcholine in vessels perfused with normal PSS (4.7 mM K^+) and those perfused with 20 mM K^+ PSS.

2.2.3. Series 3

In this series, we determined effects of ODQ and its combination with penitrem A (a maxi-K^+ channel antagonist) on vasodilation initiated by either 1-EBIO or acetyl-

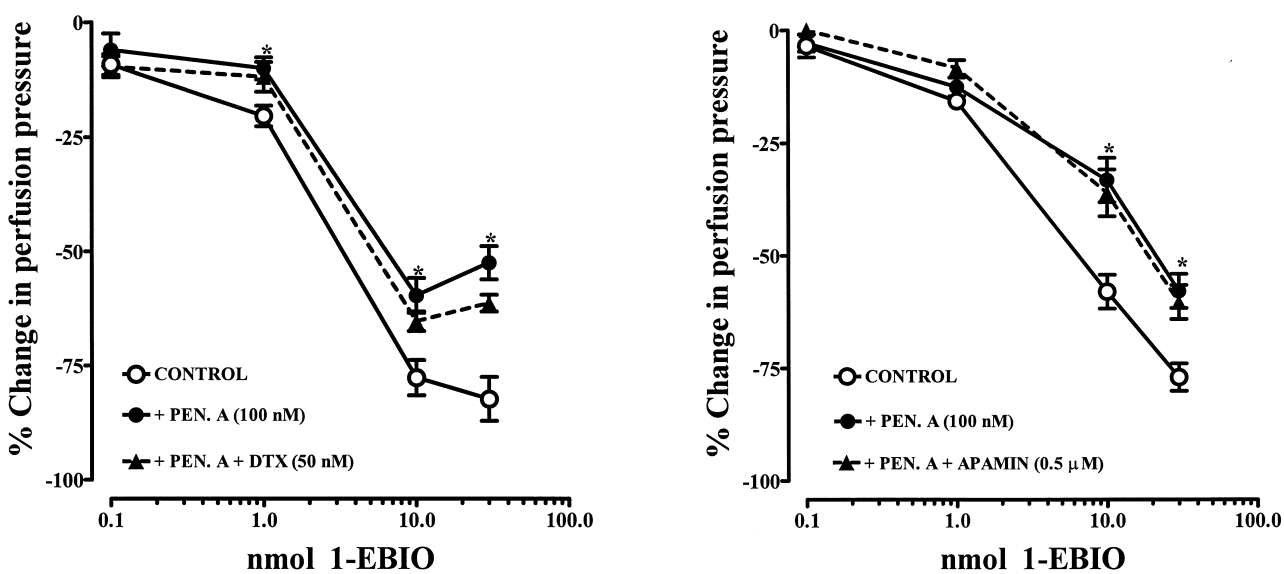


Fig. 2. Effects of K^+ channel antagonists on dilator responses initiated by 1-EBIO in isolated, perfused rat mesenteric vascular bed. In both panels, (○) represents responses to 1-EBIO alone, while (●) and (▲) represent responses to the compound in the presence of 100 nM penitrem A (Pen. A), and combinations of Pen. A with 50 nM dendrotoxin (DTX, left panel), or Pen. A with 0.5 μM apamin (right panel). Each data point on the graphs represents the mean ± S.E.M., $n = 8$. * Denote statistical difference ($P < 0.05$) between responses in the absence (control) vs. in the presence of antagonist.

choline. The experiments, carried out on vascular beds perfused with normal (with 4.7 mM K^+) PSS, involved a comparison of dose–response curves to agonists alone with those to the agonists in the presence of ODQ (10 μM), or ODQ + penitrem A (100 nM). Control experiments were performed with dimethylsulfoxide (DMSO), the vehicle for both ODQ and penitrem A. We also tested effects of ODQ and penitrem A on vasodilation initiated by sodium nitroprusside (a nitric oxide donor compound) in order to ascertain their relative specificity as nitric oxide-sensitive guanylate cyclase and maxi-K^+ channel blockers, respectively.

2.2.4. Series 4

This series of experiments investigated the influence of endothelium and of vessel perfusion pressure on vasodilation initiated by 1-EBIO. We compared dose–response curves to 1-EBIO on endothelium-intact vasculature, with those obtained in endothelium-denuded vascular beds. Endothelium was denuded from the vascular beds by infusing distilled water for 5 min. We also compared the responsiveness of intact and denuded vascular beds to acetylcholine and sodium nitroprusside in order to establish that endothelial cells were denuded, and to assess the functional integrity of underlying smooth muscle cells, respectively.

The influence of vessel tone was investigated by studying vasodilator responses to 1-EBIO (30 nmol), acetylcholine (100 pmol) and sodium nitroprusside (1 nmol) in vessels constricted to an active tonus (i.e., agonist-induced minus basal perfusion pressures) of between either 60 and 80, or 120 and 140 mm Hg. The concentration of cirazoline (α_1-adrenoceptor agonist) was titrated to produce the desired level of vessel tone.

2.3. Drugs

1-Ethyl-2-benzimidazolinone (1-EBIO), and ODQ (1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one) were purchased from Tocris Cookson, St. Louis, MO. Acetylcholine hydrobromide, apamin, atropine sulfate, chlorpheniramine maleate, L-NAME, ouabain, popranolol hydrochloride and sodium nitroprusside were obtained from Sigma, St. Louis, MO. Penitrem A and dendrotoxin were purchased from Biomol Res. Lab., Plymouth Meeting, PA, while cirazoline hydrochloride was purchased from Research Biochemicals Int., Natick, MA. 1-EBIO, penitrem A and ODQ were dissolved in DMSO; solutions of all other compounds were made in distilled water.

2.4. Data analysis

Changes in perfusion pressure were expressed as a percentage of the pressure before the administration of a vasodilator agent. Values are expressed as mean ± S.E.M. Differences between the mean values were compared using either the Student's *t*-test (single comparisons), or the Repeated Measures ANOVA (multiple comparisons). The Dunnett's multiple comparison test was used to determine differences between the means of individual groups. In all cases, differences were considered significant when $P < 0.05$.

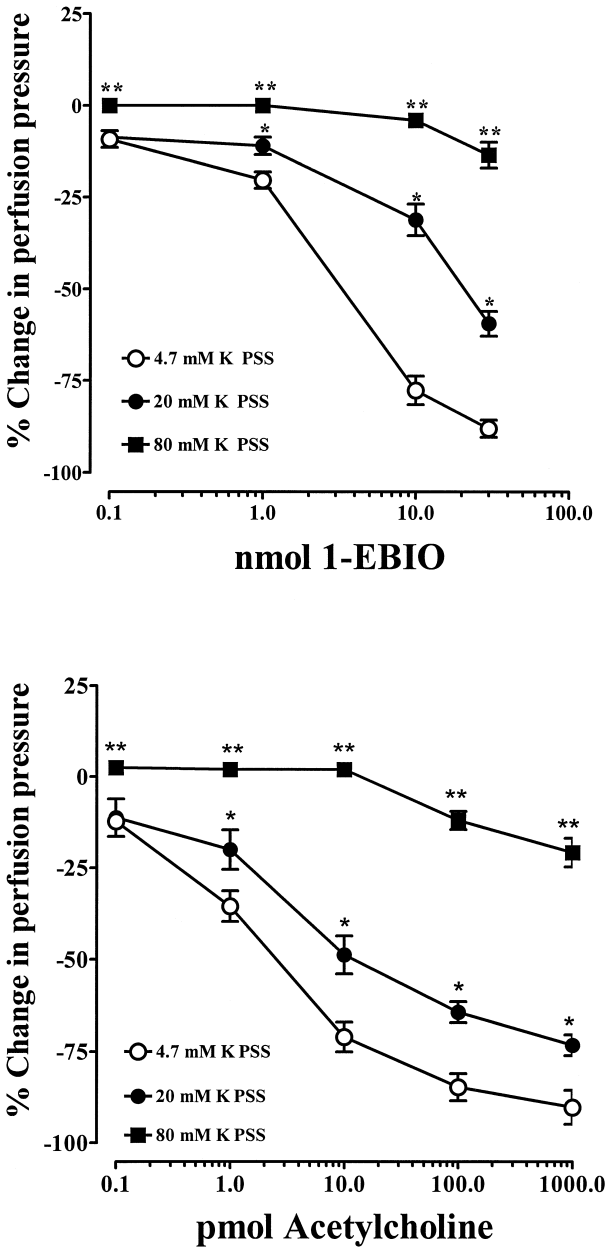


Fig. 3. Responsiveness of mesenteric vascular beds perfused with physiological salt solutions containing different amounts of KCl to 1-EBIO (top panel) and acetylcholine (lower panel). Increased vascular tone was maintained with either an infusion of 0.5–1 μM cirazoline (4.7 or 20 mM K^+ PSS), or with 80 mM K^+ PSS. Each data point on the graphs represents the mean ± S.E.M., $n = 6$. * and ** denote statistical differences between responses in 4.7 mM K^+ vs. 20 and 80 mM K^+, respectively.

3. Results

The basal perfusion pressure of mesenteric vascular beds perfused with normal PSS (i.e., 4.7 mM KCl) was 22.6 ± 0.3 mm Hg ($n = 58$). Continuous infusion of cirazoline (1 μM) initiated increases in perfusion pressure to a sustained level of 118.0 ± 2.1 mm Hg. Bolus applications of compound 1-EBIO (0.1–30 nmol), or acetylcholine (0.1–100 pmol) initiated dose-dependent decreases in perfusion pressure. Atropine (50 nM) profoundly blocked acetylcholine-induced dilator responses, but was without effect on 1-EBIO-mediated responses (Fig. 1).

3.1. Effects of K^+ channel antagonists on cirazoline-induced tone, and on 1-EBIO-mediated vasodilation

In cirazoline (1 μM) pre-constricted, endothelium-intact and -denuded vascular beds, addition of apamin (0.5 μM; a SK_{Ca}), or penitrem A (100 nM; maxi-K) channel antagonists to the perfusion medium produced significant increase in perfusion pressures (Table 1). Conversely, the addition of dendrotoxin (50 nM; a K_V channel blocker) did not alter vascular tone. Compound 1-EBIO (0.1–30 nmol) initiated a dose-dependent decrease in perfusion pressure of cirazoline-constricted mesenteric beds. Responses were only partially attenuated by penitrem A (Fig. 2). The combination of penitrem A with either apamin or dendrotoxin produced no further blockade of 1-EBIO dilator responses (see Fig. 2). Ouabain (100 μM), an inhibitor of Na^+,K^+-ATPase, did not significantly alter the decrease in perfusion pressure in response to 30 nmol 1-EBIO: 89.3% ± 3.2%, $n = 5$, (control) vs. 86.7% ± 4.1%, $n = 5$, (during ouabain infusion).

3.2. Influence of external K^+ concentration on 1-EBIO-mediated vasodilation

1-EBIO-, or acetylcholine-mediated vasodilation decreased significantly in vessels perfused with 20 mM K^+ PSS compared to those perfused with normal (4.7 mM K^+) PSS and were nearly abolished in vessels perfused with 80 mM K^+ PSS (Fig. 3). Decreases in perfusion pressure to 30 nmol 1-EBIO were 89.3% ± 3.2% (4.7 mM K^+, normal PSS), compared to 59.5% ± 3.4% and 19.0% ± 3.9% for 20 and 80 mM K^+ PSS, respectively. Vasodilator responses to 1-EBIO, as well as to acetylcholine, during perfusion of vascular beds with high (20 mM) potassium PSS, were abolished by ODQ (10 μM), or by L-NAME (100 μM) (Fig. 4). D-NAME (100 μM) did not alter 1-EBIO, or acetylcholine responses. In vessels perfused with normal PSS, inclusion of L-NAME (100 μM) weakly, but significantly attenuated responses to these agonists: 1-EBIO (30 nmol) by 21.8% ± 1.6%; acetylcholine (10 pmol) by 23.1% ± 1.9%.

3.3. Effects of ODQ and its combination with penitrem A on vasodilation to 1-EBIO and acetylcholine

Infusion of ODQ (10 μM) increased cirazoline-induced tone of endothelium-intact, but not of endothelium-denuded vascular beds (see Table 1). 1-EBIO, or acetylcholine-mediated vasodilation in vessels perfused with normal PSS, were partially, but significantly attenuated by ODQ (10 μM). The combination of ODQ and penitrem A (a maxi-K^+ channel blocker) further attenuated acetylcholine induced relaxation, but totally abolished the re-

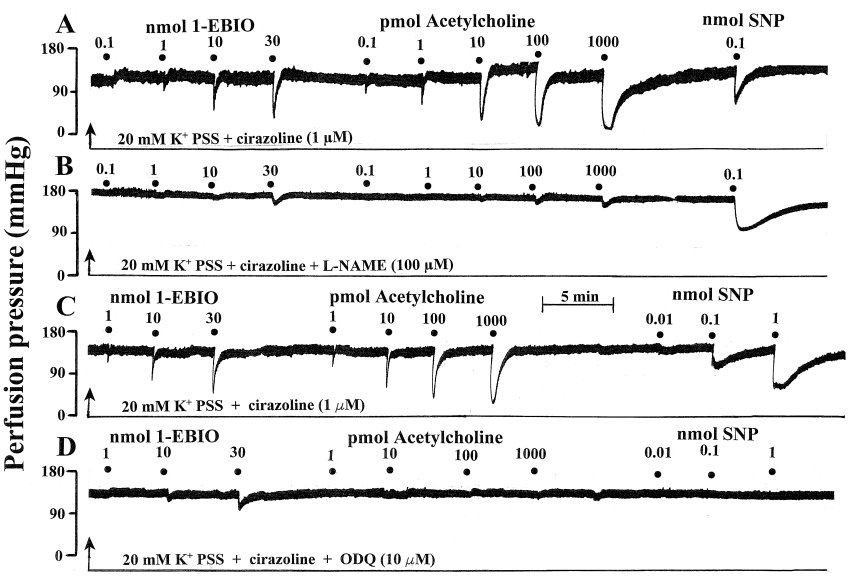


Fig. 4. Representative tracings of the dilator responses initiated by 1-EBIO, acetylcholine or sodium nitroprusside in the absence (panels A and C), and in the presence of either 100 μM L-NAME (panel B), or 10 μM ODQ (panel D). Mesenteric beds were perfused with 20 mM K^+ PSS, and vascular tone was maintained with an infusion of cirazoline (1 μM).

sponses to 1-EBIO (Fig. 5). DMSO, vehicle for ODQ and penitrem A, did not alter vasodilator effects of either 1-EBIO or acetylcholine (data not shown). ODQ (10 μM) abolished SNP-mediated vasodilation in high K^+ (Fig. 4), as well as in normal (see Fig. 5) PSS-perfused vessels. Conversely, penitrem A (100 nM) did not alter SNP-mediated vasodilation.

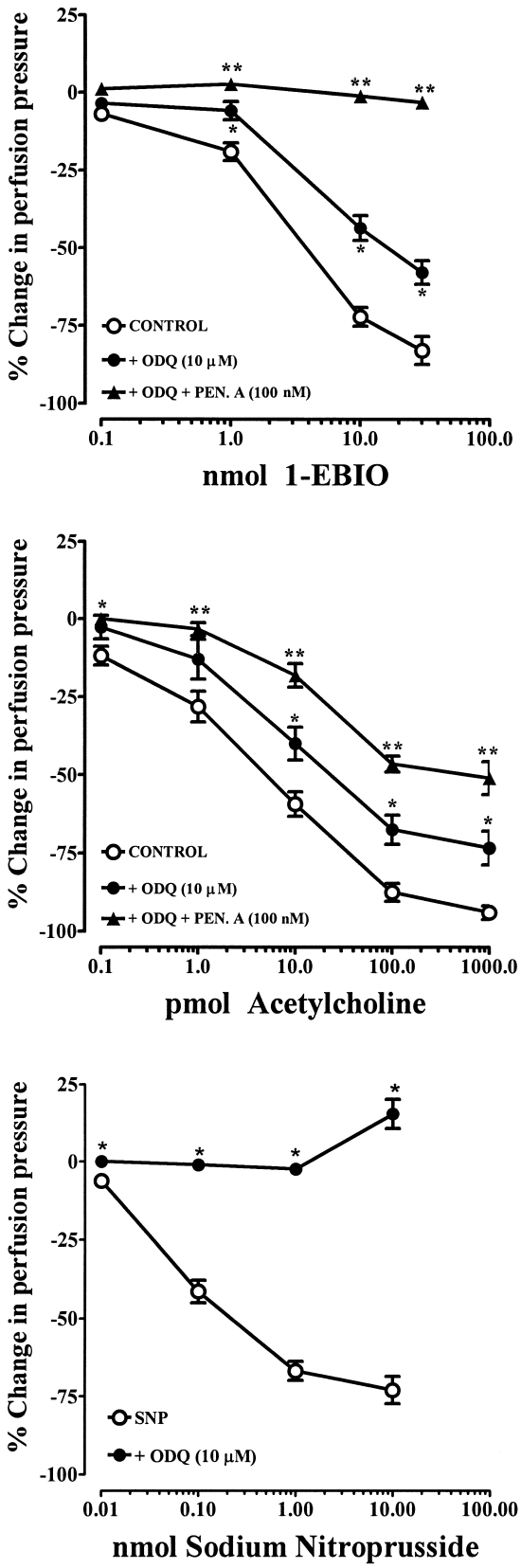


Fig. 5. Effects of ODQ (10 μM) alone (●), or in combination with penitrem A (PEN. A; ▲) on vasodilation initiated by 1-EBIO (top panel), and acetylcholine (middle panel) in mesenteric vascular beds perfused with normal PSS. The graph on the bottom panel shows complete blockade of sodium nitroprusside-mediated relaxations by ODQ (10 μM). Each data point on the graphs represents the mean ± S.E.M., $n = 5$. * and ** denote statistical differences ($P < 0.05$) between control and treated groups.

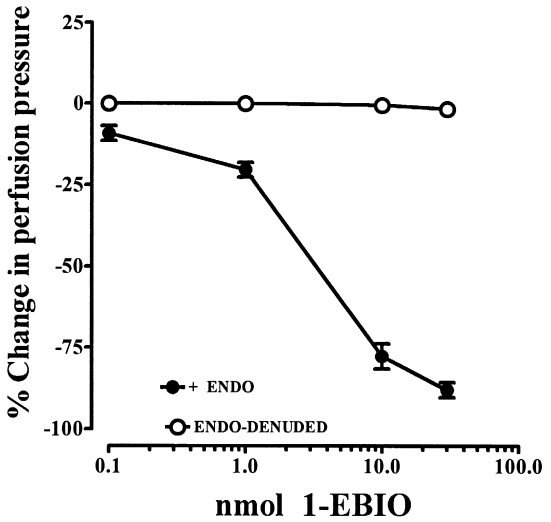
3.4. Influence of endothelium and vessel perfusion pressure on 1-EBIO-mediated vasodilation

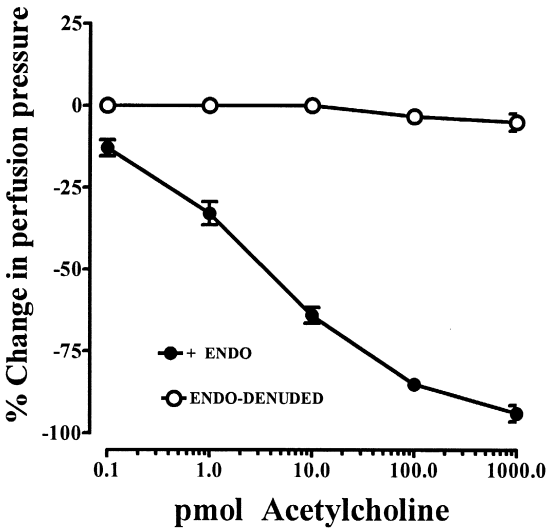
Endothelium-intact vascular beds perfused with normal PSS dilated dose-dependently to bolus applications of 1-EBIO, acetylcholine, or sodium nitroprusside. Responses to 1-EBIO and acetylcholine were abolished in vascular beds denuded of endothelium with an infusion of distilled water for 5 min (Fig. 6). The dilator response to 0.1 nmol sodium nitroprusside was significantly greater in endothelium-denuded (78.1% ± 0.9%), compared to endothelium-intact (31.7% ± 1.4%) vascular beds. Denuded, as well as vessels treated with penitrem A, apamin, or ODQ, exhibited greater increases in perfusion pressure to cirazoline. However, such increases in tone apparently did not affect responses to 1-EBIO, acetylcholine, or sodium nitroprusside. There was no significant difference in the vasodilator response to any of the agonists in vessels constricted to between 60 and 80 mm Hg and those constricted to between 120 to 140 mm Hg with 1 and 3 μM cirazoline, respectively (see Fig. 6).

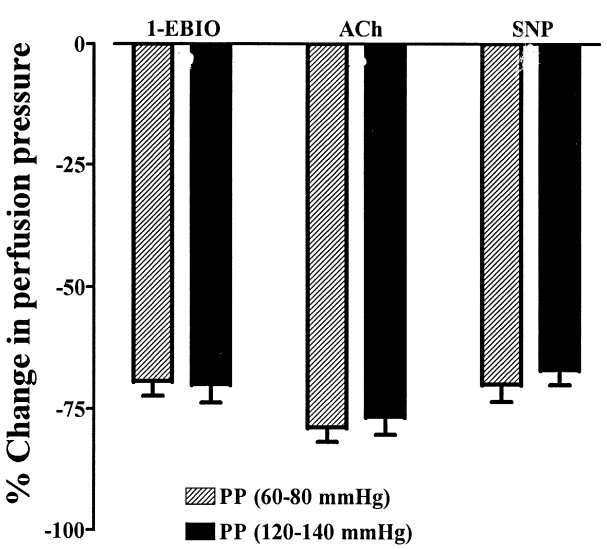
4. Discussion

Hyperpolarization of many blood vessels occurs by the opening of K_{Ca} channels. 1-EBIO is a direct activator of K_{Ca} channels in epithelial cells and thus potentially valuable for studying cellular hyperpolarization and function. Our results indicate the presence of maxi-K^+ and small-conductance (SK_{Ca}) channels, which actively contribute to the vascular tone of PSS-perfused rat mesenteric vasculature. Our study also shows that 1-EBIO dilates perfused rat mesenteric vessels through activation of endothelial maxi-K^+ channels and through nitric oxide formation.

K^+ channels are ubiquitous in their distribution in vascular tissues. Endothelial cells possess voltage-gated inwardly rectifying K^+ channels (Nilius, 1990; Laskey et al., 1992), as well as Ca^{2+}-dependent maxi-K^+, intermediate-conductance (IK_{Ca}) and SK_{Ca} K^+ channels (see Sec-







tion 1). K_{Ca}, K_V, as well as K_{ATP}, channels are present on vascular smooth muscle cells (Edwards and Weston, 1990; Kuriyama et al., 1995). In the current study, infusion of penitrem A, a maxi-K^+ channel antagonist (Knaus et al., 1994), or apamin, a SK_{Ca} channel antagonist, increased α_1-adrenoceptor agonist (cirazoline)-induced vascular tone. Our observations suggest the presence of maxi-K^+ and SK_{Ca} channels in the perfused mesenteric vessels. These channels actively contribute to moderating the increase in perfusion pressure initiated by α_1-adrenoceptor activation. Dendrotoxin, a K_V channel antagonist, did not increase perfusion pressure, thus, K_V channel type is probably not important to maintenance of mesenteric vascular tone.

Compound 1-EBIO, a benzimidazolone, has been characterized as a direct activator of maxi-K^+ channels in the basolateral membrane of T84 (epithelial) cells (Devor et al., 1996a,b). Endothelial cells are vascular equivalents of epithelial cells in non-vascular tissues. They regulate the movement of blood-borne material into the smooth muscle layer, as epithelial cells regulate (allow directional) movement of solutes across cell membranes, and from one side of the cell to the other in non-vascular tissue. Our finding that 1-EBIO-mediated vasodilation is profoundly reduced in endothelium-denuded vascular beds is intriguing and suggests that the compound either specifically interacts with endothelial K^+ channels and/or triggers the production and release of an endothelium-derived relaxing factor(s).

That 1-EBIO activates endothelial K^+ channels is supported by our finding that penitrem A, a maxi-K^+ channel antagonist (Knaus et al., 1994), caused partial attenuation of the dilator effects of the compound in endothelium-intact vessels. Moreover, the magnitude of 1-EBIO-mediated vasodilation decreased as the concentration of potassium in the PSS increased. Thus, it is reasonable to propose that 1-EBIO activates endothelial K^+ channels to initiate hyperpolarization which is opposed by the depolarizing action of increasing $[K^+]$ in the PSS. However, it is also apparent that relaxation of mesenteric beds initiated by 1-EBIO involves more than activation of K^+ channels. The remnant vasodilator responses to 1-EBIO in either 20 or 80 mM K^+ PSS were abolished by a nitric oxide synthase inhibitor, L-NAME. The D-isomer of this inhibitor does not inhibit nitric oxide synthase, nor block vasodilator effects of 1-EBIO. This suggests that a component of 1-EBIO vasodilation involves the formation of nitric oxide. Furthermore, compound ODQ, a selective inhibitor of nitric oxide-sensitive guanylate cyclase (Boulton et al., 1995; Garthwaite et al., 1995; Moro et al., 1996), partially

Fig. 6. Responses of endothelium-intact (●) and endothelium-denuded (○) mesenteric vascular beds to 1-EBIO (top panel) and acetylcholine (middle panel). The graph on the bottom panel shows the non-influence of cirazoline-induced tone on agonist-mediated vasorelaxation. Each data point on the graphs represents the mean ± S.E.M., $n = 5$.

attenuated responses obtained to 1-EBIO, or acetylcholine during infusion of normal PSS and abolished responses to the compounds in vascular beds perfused with 20 mM K^+ PSS. These results corroborate those obtained in the presence of L-NAME and further strengthens our conclusion that nitric oxide formation participates in the dilator action of 1-EBIO or acetylcholine. Ouabain, an inhibitor of Na^+,K^+-ATPase activity, did not alter the dilator action of this benzimidazolinone.

The similarity in the vasodilator profiles of 1-EBIO and acetylcholine on perfused rat mesenteric vascular bed is striking, and deserves comment. Dilator effects of both compounds were endothelium-dependent, and were not strongly blocked by modulators of nitric oxide-cyclic GMP pathway in vessels perfused with normal PSS. First, compound ODQ, a selective inhibitor of nitric oxide-sensitive guanylate cyclase, at a concentration that completely abolished dilator responses to sodium nitroprusside, only caused slight but significant attenuation of acetylcholine or 1-EBIO responses during infusion of normal PSS. This shows that guanylate cyclase activation contributes, perhaps to a smaller extent, to vasodilator actions of the two compounds. Second, responses to both agonists can be modulated with K^+ channel antagonists-penitrem A, a maxi-K^+ channel antagonist, caused partial attenuation of 1-EBIO or acetylcholine responses. The combination of this antagonist with ODQ totally blocked 1-EBIO-mediated vasodilation and caused greater attenuation of acetylcholine responses than either antagonist alone. However, 1-EBIO-, unlike acetylcholine-mediated vasodilation was not altered by atropine, a non-selective muscarinic cholinoceptor antagonist. This observation represents an important difference between the two agonists (i.e., 1-EBIO and acetylcholine) and indicates (i) 1-EBIO vasodilation does not involve muscarinic cholinoceptors, and (ii) that compound 1-EBIO and acetylcholine may respectively serve as prototypes of non-receptor and receptor-activating endothelium-dependent hyperpolarizing agonists.

In summary, the current study demonstrates that infusion of the perfused rat mesenteric vessels with a maxi-K^+ (penitrem A), or a SK_{Ca} (apamin), potassium channel antagonists produced significant increases in α_1-adrenoceptor-mediated tone. Compound 1-EBIO, a benzimidazolone, initiates endothelium-dependent decreases in perfusion pressure of the constricted rat mesenteric vessels. Responses to this compound were attenuated in part by penitrem A, and in part by L-NAME, a nitric oxide synthesis inhibitor, or by compound ODQ, a selective nitric oxide-sensitive guanylate cyclase inhibitor. The combination of penitrem A and ODQ completely abolished 1-EBIO-mediated vasodilation. We therefore conclude that (1) maxi-K^+ and SK_{Ca} channels are present in rat mesenteric vasculature, and their activities' moderate α_1-adrenoceptor-mediated tone, and (2) 1-EBIO decreases perfusion pressure by activation of endothelial maxi-K^+ channels and the formation of nitric oxide.

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