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Vasorelaxant effect of olprinone, an inhibitor of phosphodiesterase 3, on mesenteric small artery and vein of rabbits

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Abstract

The effects of olprinone, a cardiotonic agent that inhibits cyclic GMP (cGMP)-inhibited phosphodiesterase, was studied on isolated rabbit mesenteric small artery and vein. In the presence of indomethacin and propranolol, olprinone at concentrations of 10 nM to 10 μM and 1 μM to 100 μM relaxed norepinephrine-stimulated mesenteric artery and vein in a concentration-dependent manner, respectively. The relaxation was not endothelium-dependent in the artery. Removal of the endothelium, however, increased marginally the response of the vein to olprinone. Olprinone-induced relaxation was less pronounced in arteries contracted with high KCl solution + norepinephrine than in those contracted with norepinephrine alone. Nicardipine inhibited this attenuating effect of high KCl solution on the olprinone-induced relaxation. Olprinone (1 μM) enhanced the relaxation of artery and vein in response to a cAMP-increasing agent, 6-(3-dimethylaminopropionyl) forskolin (NKH477), but not to a cGMP- increasing agent, glyceryl trinitrate. Norepinephrine (10 μM) and caffeine (5 mM) elicited a transient, phasic contraction of the artery in Ca^{2+}-free solution. Both olprinone and NKH477 attenuated more potently the norepinephrine-induced contraction than the caffeine-induced contraction. When norepinephrine (10 μM) and caffeine (5 mM) were successively applied in Ca^{2+}-free solution, the contractile effect of caffeine was diminished compared to that in artery which had not been pretreated with norepinephrine. When the contraction in response to norepinephrine was partially attenuated by 1 μM olprinone, the following contraction evoked by caffeine was enlarged. It is concluded that olprinone relaxes the small artery more strongly than the vein via its direct action on smooth muscles. It is suggested that olprinone attenuates norepinephrine-induced contraction through inhibition of receptor-operated transmembrane Ca^{2+} influx and Ca^{2+} release from intracellular storage sites.

Keywords: Mesenteric small artery; Mesenteric vein; Norepinephrine; Olprinone; Phosphodiesterase 3

1. Introduction

Biochemical studies have shown the existence of cyclic GMP (cGMP)-inhibited phosphodiesterase (phosphodiesterase 3), which preferentially catalyzes the hydrolysis of cAMP, in vascular smooth muscle and cardiac muscle cells (Kariya and Dage, 1988; Lindgren et al., 1990; Komas et al., 1991; Sugioka et al., 1994). Olprinone (previous name: loprinone), an imidapyridine derivative with positive inotropic and vasodilating properties, is an inhibitor of phosphodiesterase 3, and thus, increases the cAMP content of blood vessels and hearts and enhances the response of tissues to cAMP-increasing agents (Ogawa et al., 1989; Ohoka et al., 1990; Tajimi et al., 1991; Itoh et al., 1993; Sugioka et al., 1994). Olprinone, when infused intravenously to patients with previous myocardial infarction or angina pectoris, increases left ventricular contractility and, at the same time, lowers systemic blood pressure with a decrease in total peripheral resistance (Murakami et al., 1995; Takaoka et al., 1993). In addition, the modest venodilating effect of olprinone has been demonstrated in preclinical in vivo studies with dogs (Ohhara et al., 1989; Kubota et al., 1991; Tanio et al., 1991).

Although olprinone has been suggested to improve cardiac performance not only through its inotropic but also through its vaso- (veno-) dilating effects in vivo, its direct action in isolated preparations from resistance artery and vein has not been clarified. Furthermore, the role of the endothelium on the actions of olprinone in resistance

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artery and vein remains unclear. At the moment, only data obtained with endothelium-denuded rat aorta are available (Ohoka et al., 1990; Tajimi et al., 1991; Itoh et al., 1993). Briefly, olprinone inhibited vascular contractility via a decrease in intracellular Ca^{2+} concentration and a decrease in the sensitivity of contractile elements to Ca^{2+} in rat aorta. The present study was, therefore, designed to characterize the actions of olprinone in rabbit mesenteric resistance artery and adjacent vein as follows: (1) to assess the tissue selectivity of olprinone, olprinone-induced relaxation was studied in norepinephrine-stimulated artery and vein with or without endothelium; (2) to characterize the action of olprinone, it was determined whether olprinone altered the relaxation mediated through the formation of cAMP or cGMP; and (3) since cAMP has been reported to play a role in transmembrane Ca^{2+} flux and in the release of Ca^{2+} from storage sites activated by agonist (Bülbring and Tomita, 1987; Supattapone et al., 1988; Shafiq et al., 1992; Ito et al., 1993), the effect of olprinone was examined on contractions associated with receptor-operated and voltage-dependent Ca^{2+} influx. Furthermore, the effects of olprinone were determined on norepinephrine- and caffeine-induced contractions of the mesenteric artery in Ca^{2+}-free solution.

2. Materials and methods

2.1. Vascular preparations and tension recording

Male Japan white rabbits (supplied from Kitayama Labs, Japan), weighing 1.9–2.3 kg, were anesthetized with pentobarbitone sodium (Nembutal, 40 mg/kg, i.v.) and killed by rapid exsanguination. The entire mesenterium was removed and place into cold Krebs–Henseleit bicarbonate (KHB) solution of the following composition (mM): NaCl 114, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and dextrose 10. The second-order branches of the superior mesenteric artery and the adjacent vein were cut into rings, 2 mm long, and suspended between two pins under an optimal resting tension of 300 and 150 mg, respectively, in 5 ml of warmed (37°C), oxygenated KHB solution for isometric recordings. The KHB solution contained 2 μM propranolol and 10 μM indomethacin to avoid β-adrenoceptor-mediated responses and the possible production of cyclo-oxygenase products, respectively (Fujimoto and Itoh, 1997). In some preparations, the endothelium was removed by rubbing the intimal surface with a metal wire. After an equilibration period of 60–90 min, the presence or absence of the endothelium was confirmed by the response to acetylcholine (1 μM, artery) and calcium ionophore A23187 (150 nM, vein) during a contraction elicited by norepinephrine (1 μM). The preparation used was without endothelium, unless otherwise stated in the text.

2.2. Relaxation responses to olprinone, 6-(3-dimethylaminopropionyl) forskolin (NKH477) and glyceryl trinitrate

The endothelium-intact and -denuded mesenteric arteries and veins were contracted three times with 1–10 μM norepinephrine for 10 min at 30- to 50-min intervals until the responses were reproducible. These preparations were then contracted with norepinephrine to 75–85% (EC_{75}) of the maximum norepinephrine-induced response. It has been found that mean EC75 values for norepinephrine in the endothelium-intact and -denuded tissues are 2 and 0.9 μM (artery) and are 1 and 0.2 μM (vein), respectively (Fujimoto and Itoh, 1997). After the contraction had reached steady state, two cumulative concentration–response curves for olprinone (1 nM–10 μM), NKH477 (3 nM–1 μM) and glyceryl trinitrate (1 nM–10 μM) were obtained with an interval of 90–120 min between the curves. In one of the paired preparations, the second curve for NKH477 and glyceryl trinitrate was made 30 min after and during treatment with 1 μM olprinone. Another untreated preparation was used as control. In some experiments, the mesenteric small artery was contracted with 15.9 and 35.9 mM KCl in the presence and absence of 0.3 μM nicardipine and then further contracted with norepinephrine to obtain a contraction similar to that elicited with norepinephrine (0.9 μM) alone; the concentration of norepinephrine used was varied to obtain a comparable increase in tone in each preparation. At the end of the experiment, papaverine (0.1 mM) was added to obtain the maximum relaxation. Since papaverine relaxed the preparations back to almost baseline tension, relaxation responses to the vasorelaxants are expressed as a percentage of the papaverine-induced relaxation (100%). Potencies of the drugs are expressed as negative log EC_{50} (pD_2) values, where EC_{50} is the molar concentration producing 50% of the maximum drug response in a given concentration–response curve.

2.3. Norepinephrine- and caffeine-induced contraction in Ca^{2+}-free solution containing EGTA

The endothelium-denuded mesenteric small artery was contracted with 10 μM norepinephrine three times at 50-min intervals in the normal KHB solution. Next, the preparation was exposed for 20 min to KHB solution containing olprinone (1 and 10 μM) or NKH477 (1 and 10 μM) and then contracted with 10 μM norepinephrine 2 min after the preparation was exposed to a Ca^{2+}-free solution containing 0.6 mM EGTA with olprinone or NKH477 (at the same concentrations). In another series of experiments, the preparations were contracted with 5 mM caffeine, using the same protocol as that used for norepinephrine. The contractions are expressed as percentages of the third contraction induced by norepinephrine and

caffeine in the normal KHB solution (containing 2.5 mM Ca^{2+}).

2.4. Effects of olprinone on contractions induced by successively applied norepinephrine and caffeine

Ten micromolar norepinephrine and 5 mM caffeine were successively applied in normal KHB solution to the endothelium-denuded artery for 2 min at 5-min intervals, followed by a 50-min application of the KHB solution. These procedures were repeated twice more, so that reproducible responses were obtained. Thirty minutes after the last contraction with caffeine, the artery was exposed to 1 μM olprinone in the normal KHB solution for 20 min and then in Ca^{2+}-free solution containing 0.6 mM EGTA for 2 min before the preparation was contracted with 10 μM norepinephrine for 1 min in the presence of 1 μM olprinone in Ca^{2+}-free solution. Five minutes after both norepinephrine and olprinone were washed out by adding fresh Ca^{2+}-free solution, the artery was again contracted with 5 mM caffeine (group E). In the first control group (group C1), norepinephrine and caffeine were successively applied as in the group E, but the artery was not exposed to olprinone. In some experiments (group C2), the artery was exposed to olprinone but not contracted with norepinephrine, and neither olprinone nor norepinephrine was applied to the artery in the third control group (group C3). The norepinephrine- and caffeine-induced contractions in Ca^{2+}-free solution are expressed as percentages of the corresponding third control contractions obtained in the normal KHB solution.

2.5. Drugs and solutions

The following drugs were dissolved in distilled water and diluted with the KHB solution: acetylcholine chloride (Sigma, St. Louis, MO, USA), nicardipine HCl (Sigma), (−)-norepinephrine bitartrate (Sigma), caffeine anhydrous (Sigma) and DL-propranolol HCl (Sigma). Indomethacin (Sigma) was dissolved in ethanol. The final concentrations (less than 0.1%) of this solvent in the bathing medium had no noticeable effect on muscle contraction or relaxation. The Ca^{2+}-free solution was prepared by removing $CaCl_2$ from the normal KHB solution and by adding ethyleneglycol-bis-(β-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA, Dojin, Japan) at a final concentration of 0.6 mM. Olprinone HCl was kindly provided by Eisai (Tokyo, Japan), and *N*,*N*-dimethyl-β-alanine[3*R*-(3α, 4αβ, 5β, 6β, 6*a*α, 10α, 10*a*β, 10*b*α)]-5(acetyloxy)-3-ethenyldodecahydro-10,10*b*-dihydroxy-3,4*a*,7,7,10*a*-pentamethyl-1-*oxo*-1*H*-naphthol[2,1-*b*]pyran-6-yl ester hydrochloride (NKH477) and glyceryl trinitrate were gifts from Nippon Kayaku, (Tokyo, Japan).

2.6. Statistical analysis

Results are reported as mean values ± S.E. of the number (n) of observations. All the data were analyzed by the Student's *t*-test for paired or non-paired data. Statistical significance was assumed when the P value was less than 0.05.

3. Results

3.1. Relaxation responses to olprinone, NKH477 and glyceryl trinitrate

Olprinone elicited concentration-dependent relaxations in endothelium-intact preparations of the mesenteric small artery and of the adjacent vein which had been contracted with 2 μM and 1 μM norepinephrine, respectively (Fig. 1). In Fig. 1, olprinone-induced relaxation is expressed as a percentage of the response to 0.1 mM papaverine. The absolute values for the relaxation to papaverine were 424 ± 31 mg ($n = 10$) and 310 ± 24 mg ($n = 21$) in endothelium-intact artery and vein, respectively. In endothelium-denuded artery and vein, these were 496 ± 37 mg ($n = 17$) and 362 ± 29 mg ($n = 13$), respectively. Removal of the endothelium did not alter the response to olprinone in the artery, but significantly increased the response to olprinone in the vein. The pD_2 values (M) and maximum relaxation for olprinone-induced relaxation were 7.41 ± 0.05 and 83.5 ± 4.3% in endothelium-intact and 7.26 ± 0.05 and 88.2 ± 6.0% in endothelium-denuded arteries, respectively. These were 5.68 ± 0.05 and 55.2 ± 3.7% in endothelium-intact and 5.71 ± 0.03 and 64.0 ± 2.9% ($P < 0.05$) in endothelium-denuded veins, respectively. Olprinone was

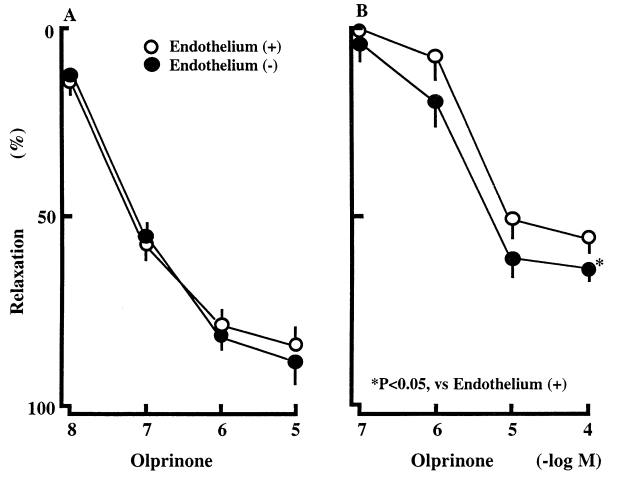

Fig. 1. Effects of olprinone on norepinephrine-induced contraction of mesenteric small artery (A) and vein (B) with and without endothelium. Ordinate, papaverine-induced relaxations are expressed as 100%. Vertical bars represent S.E. of means. ∗Significant difference from endothelium-intact vein ($P < 0.05$).

significantly more potent as a relaxant in the artery than in the vein.

NKH477 (3 nM–1 μM) concentration dependently relaxed the norepinephrine-stimulated arteries and veins. NKH477 was more potent as a relaxant in the artery than in the vein (Table 1). In both the artery and the vein, the response was potentiated by 1 μM olprinone, as assessed by the increase in the pD_2 value for the NKH477-induced relaxation (Table 1). Glyceryl trinitrate (artery: 0.3–10 μM, vein: 1–30 nM) evoked concentration-dependent relaxations in endothelium-denuded artery and vein precontracted with norepinephrine. This drug relaxed the venous preparation more markedly than the arterial preparation. Olprinone (1 μM) did not alter the response to glyceryl trinitrate (Table 1).

The relaxation response to olprinone was reduced in the artery precontracted with norepinephrine (0.70 ± 0.02 μM) in 15.9 mM KCl solution and, to a greater extent, in the artery precontracted with norepinephrine (0.34 ± 0.02 μM) in 35.9 mM KCl solution as compared to the artery precontracted with 0.9 μM norepinephrine alone in the normal KHB solution (Fig. 2A). In Fig. 2A, results are expressed as percentages of the papaverine-induced relaxation; the absolute values for relaxation in response to papaverine were 369 ± 50 mg (norepinephrine alone, $n = 7$), 320 ± 18 mg (norepinephrine in 15.9 mM KCl, $n = 8$) and 352 ± 42 mg (norepinephrine in 35.9 mM KCl, $n = 7$). In another series of experiments, the artery was contracted with 35.9 mM KCl and the contraction was completely reversed by 0.3 μM nicardipine, an L-type Ca^{2+} channel blocker. The artery was again contracted with norepinephrine (4.1 ± 0.3 μM) after the tension reached the basal level in the presence of 0.3 μM nicardipine. Under these conditions, olprinone elicited relaxations with similar concentration–response curves to those obtained with 0.9 μM norepinephrine alone and the absolute value for the relaxation in response to papaverine was 302 ± 58 mg ($n = 7$, Fig. 2A). The relaxation response of artery precontracted with 10 μM norepinephrine to olprinone, when expressed as a percentage of the response to papaverine (531 ± 32 mg, $n = 7$), was much weaker than that of artery contracted with 0.9 μM norepinephrine. For instance, the response to 10 μM olprinone was $88.2 \pm 6.0\%$ vs. $43.2 \pm 7.9\%$ ($n = 7$, $P < 0.05$) (Fig. 2B). Nicardipine (0.1–1 μM) significantly enhanced the olprinone-induced relaxation of artery precontracted with 10 μM norepinephrine (Fig. 2B). The absolute values for the response to papaverine were 173 ± 29 mg ($n = 5$) and 143 ± 9 mg ($n = 4$) in the artery contracted with norepinephrine in the presence of nicardipine at concentrations of 0.1 μM and 1 μM, respectively.

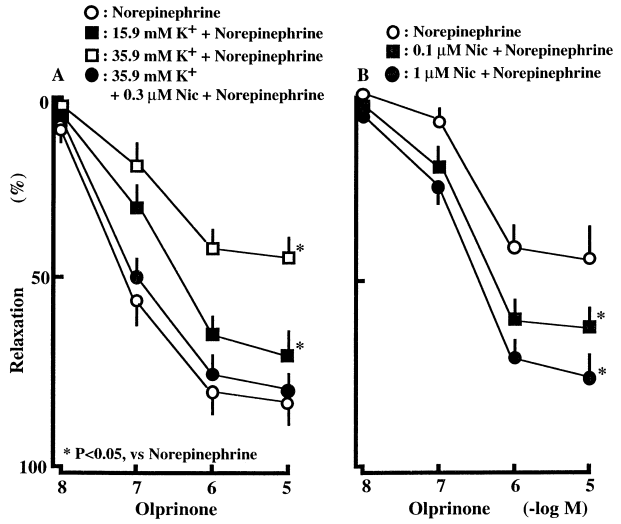

Fig. 2. (A) Concentration–response curves for olprinone-induced relaxation of endothelium-denuded artery. The tissues were contracted with norepinephrine and KCl at concentrations indicated below. ○, norepinephrine (0.9 μM) alone; ■, KCl (15.9 mM) + norepinephrine (0.70 ± 0.02 μM); □, KCl (35.9 mM) + norepinephrine (0.34 ± 0.02 μM); and ●, KCl (35.9 mM) + norepinephrine (4.1 ± 0.3 μM) in the presence of 0.3 μM nicardipine (Nic). (B) Concentration–response curves for olprinone-induced relaxation of preparations precontracted with norepinephrine (10 μM) in the absence (○) or in the presence of Nic at concentrations of 0.1 μM (■) and 1 μM (●). Ordinate, papaverine-induced relaxations are expressed as 100%. Vertical bars represent S.E. of means. * Significant difference from norepinephrine alone ($P < 0.05$).

Table 1
Relaxation responses of mesenteric artery and vein to NKH477 and glyceryl trinitrate (GTN) in the presence and absence of olprinone

	Artery	Vein
NKH477 alone:		
pD_2 value	7.47 ± 0.12	7.03 ± 0.09^a
Maximum response (%)[c]	100 ± 0	92 ± 3^a
NKH477 with 1 μM olprinone:		
pD_2 value	7.89 ± 0.08^b	$7.25 \pm 0.07^{a,b}$
Maximum response (%)[c]	100 ± 0	99 ± 3^b
GTN alone:		
pD_2 value	6.71 ± 0.09	8.83 ± 0.11^a
Maximum response (%)[c]	89 ± 2	90 ± 4
GTN with 1 μM olprinone:		
pD_2 value	6.61 ± 0.14	8.99 ± 0.1^a
Maximum response (%)[c]	85 ± 3	92 ± 5

Data are means ± S.E. ($n = 5$–7).
Significant difference from the artery[a] and corresponding control[b] ($P < 0.05$).
[c] Maximum response is expressed as a percentage of the response to 0.1 mM papaverine.

3.2. Effects of olprinone and NKH477 on norepinephrine- and caffeine-induced contractions in Ca^{2+}-free solution

The mesenteric artery was first contracted with 10 μM norepinephrine and 5 mM caffeine in the normal KHB buffer (523 ± 21 mg and 228 ± 11 mg, respectively, $n = 28$). Then the mesenteric artery was suspended for 2 min in a Ca^{2+}-free solution. Both norepinephrine (10 μM) and caffeine (5 mM) elicited a short-lived, phasic contraction in the Ca^{2+}-free solution. The peak contraction to nor-

Table 2
Effects of olprinone, NKH477 and nicardipine on norepinephrine- and caffeine-induced contractions of mesenteric artery in Ca^{2+}-free solution containing 0.6 mM EGTA

Drug	Contraction (μM)	*n*	Norepinephrine (%)	Caffeine (%)
Control		28	48.5 ± 5.5	61.4 ± 2.6
Olprinone	0.1	4	35.2 ± 6.8[a]	58.2 ± 5.3
	1	4	26.3 ± 6.3[a]	50.0 ± 2.9[a]
	10	4	18.3 ± 3.4[a]	41.5 ± 3.5[a]
NKH477	0.1	4	13.3 ± 1.2[a,b]	51.8 ± 9.2
	1	4	4.4 ± 1.5[a,b]	33.3 ± 5.5[a,b]
	10	4	0.9 ± 0.5[a,b]	20.7 ± 1.8[a,b]
Nicardipine	0.3	4	44.2 ± 2.9	57.7 ± 4.1

Results (means ± S.E.) are percentages of norepinephrine- and caffeine-induced contractions in Ca^{2+}-free solution relative to their corresponding contractions obtained in the normal KHB solution.
$P < 0.05$ compared with control[a] and with olprinone[b].

epinephrine was concentration dependently attenuated by olprinone and NKH477 (Table 2). The inhibitory effect of these agents on the caffeine-induced contraction was seen at concentrations of 1 and 10 μM, while the norepinephrine-induced contraction was significantly inhibited by these agents at 0.1 μM. NKH477 more potently inhibited the contractions induced by both agents than olprinone did (Table 2). Neither the norepinephrine-induced nor the caffeine-induced contractions were significantly affected by 0.3 μM nicardipine.

In experiments in which 10 μM norepinephrine and 5 mM caffeine were successively applied at a constant interval of 5 min in Ca^{2+}-free solution to the artery, the caffeine-induced contraction was increased when the contraction to norepinephrine had been reduced by olprinone as compared to that in the absence of olprinone (groups E vs. C1 in Table 3). Caffeine-induced contractions were significantly reduced in arteries precontracted with norepinephrine in the presence and absence of olprinone as compared to their corresponding controls (groups E vs. C2 and C1 vs. C3 in Table 3). In these experiments, the absolute values for the contractions elicited by norepinephrine and caffeine in the normal KHB buffer were 496 ± 20 mg and 212 ± 11 mg ($n = 24$), respectively.

Table 3
Caffeine-induced contraction in mesenteric artery in which norepinephrine-induced contraction had been reduced by olprinone

Group	Contraction (%)[a]	
	Norepinephrine	Caffeine
E	25.8 ± 6.5[b]	38.5 ± 4.0[b]
C1	41.4 ± 4.5[b,c]	18.5 ± 3.4[b,c]
C2	–	49.0 ± 4.3[b,c]
C3	–	54.4 ± 3.4[b,c,d]

The endothelium-denuded artery was incubated with 1 μM olprinone for 20 min in KHB solution and for another 2 min in Ca^{2+}-free solution containing 0.6 mM EGTA, and then contracted with 10 μM norepinephrine for 1 min in Ca^{2+}-free solution containing 1 μM olprinone. The artery was washed for 5 min by application of fresh Ca^{2+}-free, EGTA solution (not containing olprinone) and contracted again with 5 mM caffeine (group E). In group C1, norepinephrine and caffeine were successively applied in the same way as in group E except that olprinone was omitted. Groups C2 and C3 were treated as were groups E and C1, respectively, except that the arteries were not contracted by norepinephrine.
[a]Results (means ± S.E., $n = 6$) are percentages of norepinephrine- and caffeine-induced contractions relative to their corresponding contractions obtained in the normal KHB solution.
$P < 0.05$ compared with control contraction obtained in normal KHB solution[b] and with groups E[c] and C1[d].

4. Discussion

Although olprinone is known to dilate peripheral artery and vein in vivo (Ohhara et al., 1989; Kubota et al., 1991; Murakami et al., 1995; Takaoka et al., 1993), direct effects of olprinone on resistance arteries and veins have received far less attention than its effects on blood pressure or blood flow in intact vascular beds. The present results for rabbit mesenteric small artery and adjacent vein demonstrated that in the presence of propranolol and indomethacin, olprinone indeed evoked relaxation of these blood vessels preconstricted with norepinephrine, as reported for rat aorta (Tajimi et al., 1991; Itoh et al., 1993). The rabbit mesenteric small artery was 10-times more responsive to olprinone than was the rat aorta. In the small artery, olprinone caused relaxation at concentrations higher than 10 nM. These concentrations are close to plasma concentrations (70–200 nM) achieved after administration of clinical doses of olprinone in humans (Murakami et al., 1995) and to the concentrations (10–30 nM) of olprinone required to inhibit phosphodiesterase 3 in vitro (Ogawa et al., 1989; Sugioka et al., 1994). However, in anesthetized dogs, vaso- and veno-dilating effects of olprinone were obtained at plasma concentrations as high as 0.2–2 μM (Ohhara et al., 1989; Tanio et al., 1991). Although the vein was less sensitive to olprinone than the artery, the decreased sensitivity cannot be generalized to other phosphodiesterase 3 inhibitors in different species, i.e., pimobendan and milrinone, because in rats the mesenteric vein was more sensitive to these drugs than was the aorta (Fujimoto, 1994). The tissue selectivity of these drugs may be in part related to their relative selectivity for phosphodiesterase isozymes. For instance, in human and guinea-pig hearts olprinone is 300–400-times more selective for phosphodiesterase 3 than for phosphodiesterase 1 and 30–150-times more selective than for phosphodiesterase 2 (Ogawa et al., 1989; Sugioka et al., 1994). In rat aorta, the drug is 70-times more selective for phosphodiesterase 3 than for phosphodiesterase 1 (Itoh et al., 1993). Milrinone is 10–100-times more selective for phosphodiesterase 3 than for other types of phosphodiesterase (Ito et al., 1988; Kariya and Dage, 1988). In contrast, pimobendan is only 3-times more selective for phosphodiesterase 1 relative to phosphodiesterase 3 (Itoh et al., 1993). To date, the distribution of

phosphodiesterase isozymes in small arteries and veins has not been studied. Therefore, the present results do not exclude the possibility that the tissue selectivity of these drugs may be due to the heterogeneous distribution of the enzymes in these tissues. Furthermore, there might be tissue differences in relaxation mechanisms activated by cAMP, although the response of mesenteric vein to NKH477 was less pronounced than that of mesenteric artery.

When the endothelium was removed from the mesenteric artery, the response to olprinone was unchanged. This is in accordance with previous results (Kauffman et al., 1987; Schoeffter et al., 1987; Komas et al., 1991; Fujimoto, 1994; Shiraishi et al., 1998), where certain types of phosphodiesterase 3 inhibitors, such as cilostamide, cilostazol, pimobendan and milrinone, were used to induce relaxation in endothelium-denuded vascular preparations. The present results, together with the earlier findings (Lugnier and Schini, 1990), suggest that phosphodiesterase 3 activity may be absent in endothelial cells in rabbit mesenteric resistance artery. In the vein, removal of the endothelium resulted in a slightly but significantly enhanced response to olprinone. The biological significance of this enhancement remains an open question, although it may reflect differences in the concentrations of norepinephrine used (endothelium-intact and -denuded, 1 and 0.2 μM, respectively). These results suggest that olprinone mainly acted on vascular smooth muscle cells to cause relaxation (Komas et al., 1991; Tajimi et al., 1991; Itoh et al., 1993).

It has been found that olprinone selectively inhibits phosphodiesterase 3, which preferentially catalyzes the hydrolysis of cAMP (Ogawa et al., 1989; Itoh et al., 1993; Sugioka et al., 1994), suggesting that the vasorelaxing effects of olprinone are mediated by the intracellular actions of cAMP, possibly via activation of cAMP-dependent protein kinase (Ohoka et al., 1990; Honda et al., 1994), although the participation of cGMP-dependent protein kinase in cAMP-mediated relaxation has also been proposed (Eckly-Michel et al., 1997). If this is so, it can be expected that olprinone enhances the effect of cAMP-forming drugs. Indeed, we found that olprinone enhanced the vasorelaxation in response to NKH477 but not to glyceryl trinitrate, which produces cGMP-mediated relaxation.

It has been shown that the vasorelaxation in response to phosphodiesterase 3 inhibitors is more pronounced in agonist-stimulated arteries than in arteries depolarized by high KCl solution (Fujimoto and Matsuda, 1990; Tajimi et al., 1991; Itoh et al., 1993; Shiraishi et al., 1998). We, therefore, examined the following possibilities: (1) olprinone inhibits more markedly contractions associated with agonist-operated Ca^{2+} influx than those that are associated with voltage-dependent Ca^{2+} influx, and (2) olprinone modifies the relaxation by inhibiting agonist-induced Ca^{2+} release from intracellular storage sites. As shown in Fig. 2, the contraction in response to norepinephrine (0.7–0.34 μM) in arteries depolarized with high KCl solution (15.9–35.9 mM) was more resistant to the inhibitory effect of olprinone than the contraction elicited by norepinephrine (0.9 μM) alone. In addition, nicardipine at 0.3 μM, which completely prevented high K^+ (35.9 mM)-induced contractions, enhanced the relaxation elicited by olprinone in the artery contracted with norepinephrine (4 μM) plus KCl (35.9 mM). In another series of experiments, arteries were contracted with norepinephrine at 10 μM in the presence of different concentrations of nicardipine (0.1 and 1 μM). Nicardipine partially inhibited the contraction elicited by norepinephrine and the remaining contraction was largely inhibited by olprinone. This suggests that membrane depolarization itself did not significantly affect the olprinone-induced relaxation. Moreover, it appears that the artery contracted as a result of activation of receptor-operated Ca^{2+} influx was more susceptible to the inhibitory effect of olprinone than that contracted as a result of depolarization-induced Ca^{2+} influx. Alternatively, an increase in intracellular Ca^{2+} concentrations due to high KCl solution may have been responsible for the decrease in the inhibitory effect of olprinone.

Cyclic AMP attenuates agonist-induced increases in intracellular Ca^{2+} concentration through inhibition of agonist-induced release of Ca^{2+} from intracellular storage sites, enhancement of Ca^{2+} uptake into the storage sites and activation of Ca^{2+} extrusion into the extracellular space (Bülbring and Tomita, 1987; Supattapone et al., 1988; Shafiq et al., 1992; Ito et al., 1993). In addition, cAMP affects myofilament Ca^{2+}-sensitivity in vascular smooth muscles (Shafiq et al., 1992; Shiraishi et al., 1998). In Ca^{2+}-free solution, both norepinephrine (10 μM) and caffeine (5 mM) elicited a short-lived, phasic contraction in mesenteric small artery, and the contraction remained unchanged in the presence of 0.3 μM nicardipine, indicating that these contractions are due to the release of Ca^{2+} from storage sites, although the mechanisms underlying the release of Ca^{2+} are different between norepinephrine and caffeine (Itoh et al., 1992). The contractions elicited by norepinephrine and caffeine in Ca^{2+}-free solution were attenuated by olprinone. The norepinephrine-induced contraction was more sensitive to olprinone than was the caffeine-induced contraction. Furthermore, the caffeine-induced contraction was reduced in preparations contracted by norepinephrine in Ca^{2+}-free solution (Table 3, C1 vs. C2), suggesting that the two agents acted via the same Ca^{2+}-storage sites. The larger contraction elicited by caffeine in arteries in which the norepinephrine-induced contraction was diminished by olprinone (Table 3, E vs. C1) may suggest that olprinone reduces the release of Ca^{2+} elicited by norepinephrine and thereby increases the amount of Ca^{2+} remaining in the stores that are responsive to caffeine. These results indicate that olprinone had a more selective inhibitory effect against the norepinephrine-activated contractile pathway proximal to Ca^{2+} release. Alternatively, olprinone might inhibit the norepinephrine-

induced increase in myofilament Ca^{2+}-sensitivity (Nishimura et al., 1988; Shiraishi et al., 1997).

In conclusion, olprinone at clinically relevant concentrations potently inhibited the norepinephrine-induced contraction of the mesenteric small artery of the rabbit. The agent was, to a lesser extent, a venorelaxant. The effect was endothelium-independent in the artery, and the agent was not a β-adrenoceptor agonist nor an inhibitor of voltage-dependent Ca^{2+} influx. The agent potentiated the relaxation elicited by a cAMP-elevating agent (NKH477), but not that elicited by a cGMP-elevating agent (glyceryl trinitrate). The effect of olprinone is suggested to be accounted for by cAMP-mediated inhibition of contractile mechanisms initiated by receptor-operated Ca^{2+} influx and by the release of Ca^{2+} from norepinephrine-sensitive storage sites in vascular smooth muscles.

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