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European Journal of Pharmacology 528 (2005) 137–143
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Effects of a phosphodiesterase 3 inhibitor, olprinone, on rhythmical change in tension of human gastroepiploic artery

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Received 9 June 2005; received in revised form 14 October 2005; accepted 26 October 2005

Abstract

The gastroepiploic artery, used widely as a conduit in coronary artery bypass grafting, has high vasospasticity. The aims of this study were to examine the vasorelaxant effects of three phosphodiesterase 3 (PDE3) inhibitors, olprinone, milrinone and amrinone, on isolated gastroepiploic arterial preparations in comparison with a calcium channel blocker diltiazem, and to confirm the mRNA expression of PDE3A isoenzyme using reverse transcription–polymerase chain reaction (RT-PCR) in the human gastroepiploic artery isolated from stomach removed in cancer surgery. In endothelium-denuded gastroepiploic arterial preparations, phenylephrine (100 μM) produced spontaneous, rhythmical changes in tension consisting of repeated contraction and relaxation. Olprinone at a concentration of 10 μM ($n=6$) significantly inhibited the frequency (2.7±1.1 times/30 min vs. 6.2±0.7 times/30 min in the vehicle group), maximum tension (1.7±0.6 g vs. 3.6±0.6 g in the vehicle group) and minimum tension (0.6±0.2 g vs. 1.7±0.3 g in the vehicle group) of rhythmical changes. Such potency is comparable to that of diltiazem, but is stronger than milrinone and amrinone. RT-PCR using PDE3A- or PDE3B-specific oligonucleotide primer demonstrated the existence of PDE3A sequence in the gastroepiploic artery. These results suggest that olprinone, a potent PDE3A inhibitor, would be suitable for protecting against perioperative spasm during coronary artery bypass graft surgery.

Keywords: Gastroepiploic artery; Phosphodiesterase inhibitor; Olprinone; Diltiazem; Phosphodiesterase 3A; Phenylephrine

1. Introduction

Currently, the gastroepiploic artery is one of the promising alternatives in coronary artery bypass grafting, together with the internal mammary artery, the radial artery and the saphenous vein (Reardon et al., 1997). However, the gastroepiploic arterial grafts cause major vasospasm, which limits the maintenance of blood flow through bypass conduits, especially in the arteries during the perioperative and/or postoperative period. Perioperative or postoperative spasm is the greatest problem and requires urgent treatment, since it increases the risk of morbidity and mortality (Mills and Everson, 1989; Suma, 1990). Although the exact mechanism is unknown, perioperative spasm has generally been attributed to exposure to hypoxia and endothelial injury during isolation and preparation of the graft materials (Hashimoto et al., 1991). Graft spasms are believed to be triggered by the factors released during operational ischemic intervals and by endothelial injury (Hashimoto et al., 1992; Akar et al., 1994). Accordingly, a new, improved type of antispastic therapy is needed to lessen the risk of arterial narrowing and occlusion, and to expand the opportunities to use the gastroepiploic artery for coronary artery bypass grafting.

Phosphodiesterase 3 (PDE3) isoforms, so-called cGMP-inhibited PDEs, exhibit high affinity for cAMP. cDNA for two PDE3 isoforms cloned from human cardiac and rat adipocyte cDNA libraries indicated the presence of two distinct, but related genes, PDE3A and 3B, respectively (Meacci et al., 1992; Taira et al., 1993). PDE3A, is a 120 kDa protein found in myocardium and platelets, and PDE3B is a 135 kDa protein found in lymphocytes (Liu and Mauruce, 1998; Sheth et al., 1997).

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doi:10.1016/j.ejphar.2005.10.047

Olprinone (1,2-dihydro-5-(imidazo[1,2-*a*]pyridin-6-yl)-6-methyl-2-oxo-3-pyridine carbonitrile hydrochloride monohydrate) is one of the potent cardiotonic and vasodilating PDE3 inhibitors that includes amrinone and milrinone, and is an intravenous remedy for acute heart failure (Ohhara et al., 1989; Adachi and Tanaka, 1997). The IC_{50} values of olprinone and milrinone for PDE3 activity were 0.63 and 0.76 μM, respectively (Ogawa et al., 1989). The IC_{50} value of olprinone was about 30 times lower than that of amrinone, 19.5 μM (Kariya et al., 1982). We have previously reported that olprinone relaxed the phenylephrine-induced contraction of endothelium-denuded strips prepared from the human radial artery through inhibition of PDE3 isozyme activity (Adachi et al., 2000).

The objectives of the present study were to examine the vasorelaxant effects of three PDE3 inhibitors, olprinone, milrinone and amrinone, on isolated gastroepiploic arterial preparations in comparison with a calcium channel blocker, diltiazem, and to confirm the mRNA expression of PDE3A isoenzyme using reverse transcription–polymerase chain reaction (RT-PCR) in the human gastroepiploic artery isolated from stomach removed in cancer surgery. In this study, phenylephrine was selected as the standard vasoconstrictor to produce spasm in vitro, because it evokes contraction of both arteries and veins for myocardial revascularization through alpha adrenoceptor stimulation (Thorin-trescases et al., 1993; Liu et al., 1997; Adachi et al., 2000). Diltiazem was used as a representative vasodilator to prevent spasm in coronary artery bypass grafting (Acar et al., 1992).

2. Materials and methods

2.1. Vasorelaxant effects on human GEA

The procedures and handling of human tissue were approved by the Human Ethics Committee of the Osaka Medical Center for Cancer and Cardiovascular Diseases (Osaka, Japan). Written informed consent was given by each patient or each patient's family.

Segments of freshly prepared human gastroepiploic artery were removed from 13 patients with stomach cancer in the Osaka Medical Center for Cancer and Cardiovascular Diseases. The isolated gastroepiploic arterial preparations were immersed in cold physiological salt solution in an iced carrier box and transported to Eisai Tsukuba Research Laboratories the morning after the day of operation by courier.

The arterial preparations were washed with saline and placed in Krebs–Henseleit solution that contained (mM): NaCl, 118.4; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.3; KH_2PO_4, 1.2; $NaHCO_3$, 5.0; glucose, 11.0. The strips were carefully cleaned of fat and extraneous tissue, cut transversely (2–3 mm) and longitudinally, and denuded of endothelium by gentle rubbing with a swab stick. The preparations were clipped at each end with a pair of stainless steel holders connected to cotton thread and mounted in organ baths containing 10 ml of Krebs–Henseleit solution. Organ bath solutions were maintained at 37 °C and continuously aerated with 95% O_2 and 5% CO_2. The upper thread of each preparation was suspended from an isometric force transducer (TB-611T, Nihon Kohden, Tokyo, Japan). The changes in arterial smooth muscle tension were recorded isometrically via an amplifier (AP-621G, Nihon Kohden). The initial resting tension of each preparation was set at 2 g.

The preparations were washed several times with Krebs–Henseleit solution. Then, organ baths were refilled with 10 ml of Krebs–Henseleit solution and 250 μl of 2 M KCl was added to depolarize and contract the preparations. The depolarization step was repeated from four to six times. Then, the preparations were initially contracted by an addition of 100 μl of 10 mM phenylephrine (Sigma, St. Louis, MO, USA). Subsequently, organ baths were filled with 10 ml of Krebs–Henseleit solution and 10 μl of 10 mM *N*ω-nitro-L-arginine methyl ester (Sigma), and 10 μl of 10 mM indomethacin (Sigma) were added. The preparations were incubated for 15 min, then 100 μl of 10 mM phenylephrine was added into the organ bath to elicit a spontaneous, rhythmical contractile response consisting of repeated contraction and relaxation. When the rhythmical changes had stabilized over 30 min, olprinone solution was added into the organ bath to reach a final concentration of 1 or 10 μM. At the end of the experiment, 100 μl of 10 mM papaverine (Sigma) was added to obtain the maximum relaxation. The relaxation obtained in the presence of papaverine was defined as 0 g tension. The frequency for 30 min of rhythmical contractile response (time/30 min), and the maximum and minimum tensions (g) after treatment of drugs and the vehicles were measured.

Experiments were divided into two parts due to the use of different solvents. Olprinone or diltiazem was resolved to be 10 mM in distilled water as the original solution in Experiment 1. Milrinone and amrinone were prepared to be 10 and 100 mM, respectively, in 100% ethanol as the original solution in Experiment 2.

All values are shown as the mean±S.E.M. determined from the results of 6 examples in all groups, except the vehicle (ethanol)-treated group ($n=7$) in Experiment 2. The values for analyzed parameters, including the frequency of rhythmical contractile response and the maximum and minimum tensions in the olprinone, milrinone, olprinone or diltiazem-treated group, were compared with those obtained in each vehicle-treated group by the unpaired Student's *t*-test. *P* values of less than 0.05 (two-sided) were considered significant. Statistical analysis was conducted using the software package, SAS 6.12 (SAS Institute Japan Ltd., Tokyo).

2.2. RT-PCR analysis of PDE3A and PDE3B

The cDNA sequences of PDE3A and PDE3B were obtained from GeneBank (accession numbers NM000921 and NM000922, respectively). PDE3A primers were 5′-ACTC-GGACACCAAGTCGAACA-3′ (forward) and 5′-CCAGG-TTATCCATGACAAGAGGTT-3′ (reverse; corresponding to nucleotide numbers 1825–1845 and 2024–2047 in the open-reading frame of human PDE3A, respectively); the expected size of the glyceraldehyde-3-phosphate dehydrogenase (PCR) product was 223 bp. PDE3B primers were 5′-

CTGACTGATCCAAGCCTTCCA-3′ (forward) and 5′-ACAGGTAGCAATCCTGAAGTTCCT-3′ (reverse; corresponding to nucleotide numbers 1099–1119 and 1326–1349 in the open-reading frame of human PDE3B, respectively); the expected size of the PCR product was 251 bp. Primers that amplified a 226 bp cDNA for the GAPDH were included to control reaction efficiency and variations in tissue in the original RT reaction. The accession number of GAPDH was M33197, and the primers were 5′-GAAGGTGAAGGTCGGAGTC-3′ (forward) and 5′-GAAGATGGTGATGGGATTTC-3′ (reverse; corresponding to nucleotide numbers 66–84 and 272–291 in the open-reading frame of human GAPDH, respectively).

Frozen gastroepiploic arteries were thawed on ice, and total RNA was isolated using TriZol reagent (Invitrogen Corp., Carlsbad, CA), a commercially available mixture of phenol and guanidine isothiocyanate. The total RNA concentration and purity of the RNA were determined by measurement of the optical densities at 260 nm and 280 nm. A ratio of >1.7 for A260/280 was required for these studies. cDNA was synthesized from total RNA (5 μg) using oligo-d(T)16 cellulose to prime 1.25 units of multi-scribe reverse transcriptase and 0.4 units of RNase inhibitor, 5.5 mM $MgCl_2$, 500 μM dNTP, in 50 μl of reaction mixture. Reversibly transcribed cDNA (5 μl) was amplified by PCR under the following reaction mixture conditions: 4 mM $MgCl_2$, 200 μM dNTP and 0.25 units *Taq* DNA polymerase (Amplify Gold), 1× reaction buffer (Applied Biosystems, Foster, CA) containing 500 mM KCl, 100 mM Tris–HCl (pH 8.0), 10 pmol of each PCR primer. The reaction mixture was brought to a final volume of 50 μl with nuclease-free water and amplified with a thermal cycler (GeneAmp™ PCR System 9700, Applied Biosystems). The amplification of cDNA products involved denaturing at 94 °C for 30 s, annealing at 57 °C for 1 min and extension at 72 °C for 1 min. The PCR product were separated by electrophoresis in 2% agarose gel in the presence of ethidium bromide and visualized by an Ultraviolet Ray Irradiation DT-20LCP System (ATTO, Tokyo).

2.3. Reagents

Olprinone hydrochloride (MW: 304.8) was synthesized at the Department of Organic Chemistry (Eisai, Ibaraki, Japan). Diltiazem hydrochloride (MW: 451.0), milrinone (MW: 211.2) and amrinone (MW: 187.2) were purchased from Sigma.

3. Results

3.1. Vasorelaxant effects on human gastroepiploic artery

3.1.1. Spontaneous rhythmical change in tension

Phasic contraction caused by phenylephrine was followed by spontaneous, rhythmical changes in tension consisting of repeated contraction and relaxation in endothelium-denuded gastroepiploic arterial preparations (Fig. 1). There were no statistically significant differences in weight of preparation, maximum tension induced by phenylephrine, frequency of rhythmical contractile response, and maximum and minimum tensions of spontaneous rhythmical change in tension of isolated human gastroepiploic artery before drug treatment among five groups in Experiments 1 and 2 (Tables 1 and 2, respectively). The cycle numbers of rhythmical change before vehicle treatment were 6.0±0.6 times/30 min (n=6) and 4.9±0.4 times/30 min (n=7) in the vehicle groups of Experiments 1 and 2, respectively.

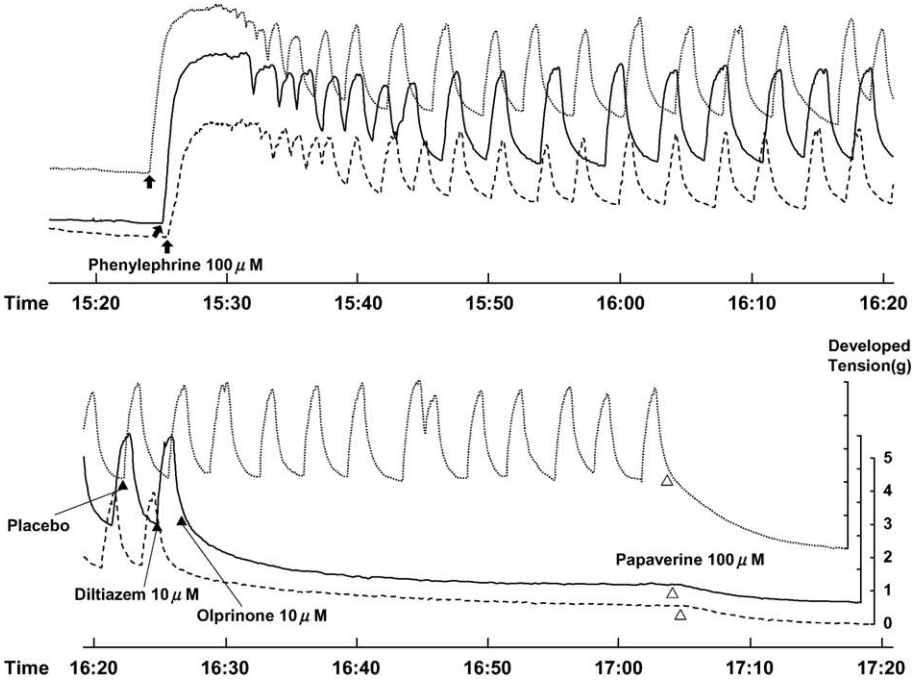

Fig. 1. Typical recordings of the effects of olprinone and diltiazem on rhythmic contractions produced by phenylephrine (100 μM) in human gastroepiploic arterial preparations without endothelium.

Table 1
Comparison of spontaneous rhythmical change in tension of isolated human gastroepiploic artery before treatment with drug

Group	Weight of preparation (mg)	Maximum tension by phenylephrine (g)	Rhythmical change		
			Frequency (times/30 min)	Maximum tension (g)	Minimum tension (g)
Placebo (n=6)	9.1±1.1	4.1±0.6	6.0±0.6	3.7±0.6	1.8±0.3
Olprinone 1 μM (n=6)	9.2±1.2	3.4±0.7	5.5±0.9	3.1±0.7	1.5±0.4
Olprinone 10 μM (n=6)	8.8±1.1	3.9±0.8	6.5±0.6	3.5±0.8	1.7±0.3
Diltiazem 1 μM (n=6)	9.3±1.5	3.9±0.4	5.5±0.6	3.7±0.4	1.7±0.2
Diltiazem 10 μM (n=6)	9.2±1.4	3.7±0.6	6.0±0.8	3.5±0.7	1.6±0.2

All values are shown as the mean±S.E.M. determined from the results of 6 examples in all groups. Neither parameter in the olprinone- or diltiazem-treated group significantly differed from that in the vehicle-treated group.

3.1.2. Frequency of rhythmical contractile response after drug treatment

The frequency of rhythmical contractile response after treatment with olprinone and diltiazem, or milrinone and amrinone in preparations precontracted with phenylephrine is shown in Fig. 2A and B, respectively. In comparison with 6.2±0.7 times/30 min (n=6) in the vehicle group of Experiment 1, the frequency after olprinone 10 μM was significantly decreased to 2.7±1.1 times/30 min. The higher concentration of diltiazem also significantly inhibited contractions, with a frequency of 0.2±0.2 times/30 min. However, the lower concentration of diltiazem tended to increase the frequency.

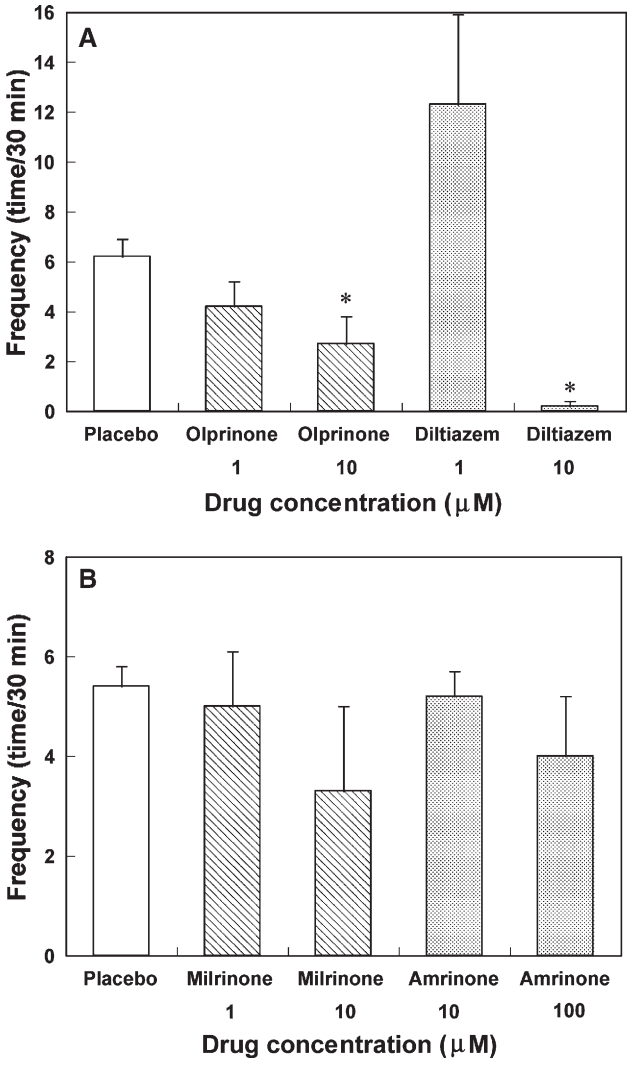

Fig. 2. (A) Effects of olprinone (1 and 10 μM) and diltiazem (1 and 10 μM) on the frequency for 30 min of rhythmical contractile response induced by phenylephrine (100 μM) in human gastroepiploic arterial preparations without endothelium (Experiment 1). (B) Effects of milrinone (1 and 10 μM) and amrinone (10 and 100 μM) on the frequency for 30 min of rhythmical contractile response induced by phenylephrine (100 μM) in human gastroepiploic arterial preparations without endothelium (Experiment 2). All values are shown as the mean±S.E.M. determined from the results of 6 examples in all groups except the vehicle (ethanol)-treated group (n=7) in Experiment 2. *P<0.05 vs. vehicle-treated group.

Table 2
Comparison of spontaneous rhythmical change in tension of isolated human gastroepiploic artery before treatment with drug

Group	Weight of preparation (mg)	Maximum tension by phenylephrine (g)	Rhythmical change		
			Frequency (times/30 min)	Maximum tension (g)	Minimum tension (g)
Placebo (n=7)	8.4±0.5	2.7±0.3	4.9±0.4	2.3±0.4	0.9±0.2
Milrinone 1 μM (n=6)	8.6±0.9	3.2±0.8	4.5±0.6	2.7±0.8	1.2±0.4
Milrinone 10 μM (n=6)	8.2±1.0	3.4±0.7	5.2±0.5	2.9±0.6	1.3±0.3
Amrinone 10 μM (n=6)	8.9±0.8	3.3±0.7	5.0±0.5	3.0±0.6	1.3±0.3
Amrinone 100 μM (n=6)	8.4±0.7	3.2±0.8	5.2±0.3	2.9±0.7	1.3±0.3

All values are shown as the mean±S.E.M. determined from the results of six examples in four groups except the vehicle (ethanol)-treated group (n=7). Neither parameter in the milrinone- or amrinone-treated group was significantly different from that in the vehicle-treated group.

On the other hand, neither milrinone 1 or 10 μM, nor amrinone 10 or 100 μM significantly affected the frequency of rhythmical contractile response in Experiment 2 (Fig. 2B).

3.1.3. Maximum and minimum tensions after drug treatment

The maximum and minimum tensions after vehicle treatment were 3.6±0.6 and 1.7±0.3 g, respectively, in Experiment 1. Olprinone 10 μM and diltiazem 1 and 10 μM significantly depressed the maximum tension to 1.7±0.6, 1.9±0.2 and 0.9±0.4 g, respectively, as indicated in Fig. 3A. Olprinone 10 μM and diltiazem 10 μM also significantly

decreased the minimum tension to 0.6±0.2 and 0.6±0.2 g, respectively (Fig. 4A).

On the other hand, although the higher concentration (100 μM) of amrinone significantly decreased the maximum and minimum tensions to 1.0±0.4 and 0.3±0.1 g, respectively, both milrinone 1 and 10 μM and amrinone 10 μM had no influence on either tensions in Experiment 2 (Figs. 3B and 4B).

3.2. Demonstration of PDE3A mRNA in human gastroepiploic artery tissue by RT-PCR

Expression of PDE3A and PDE3B was investigated by RT-PCR using PDE3A- and PDE3B-specific primers. Human gastroepiploic arterial tissue exhibited the cardiac prototype PDE3A (Meacci et al., 1992), but not the adipocyte prototype PDE3B (Taira et al., 1993). Namely, PCR yielded a band at 223 bp for the primer pair PDE3A, but no band was detected with the primer pair PDE3B (Fig. 5).

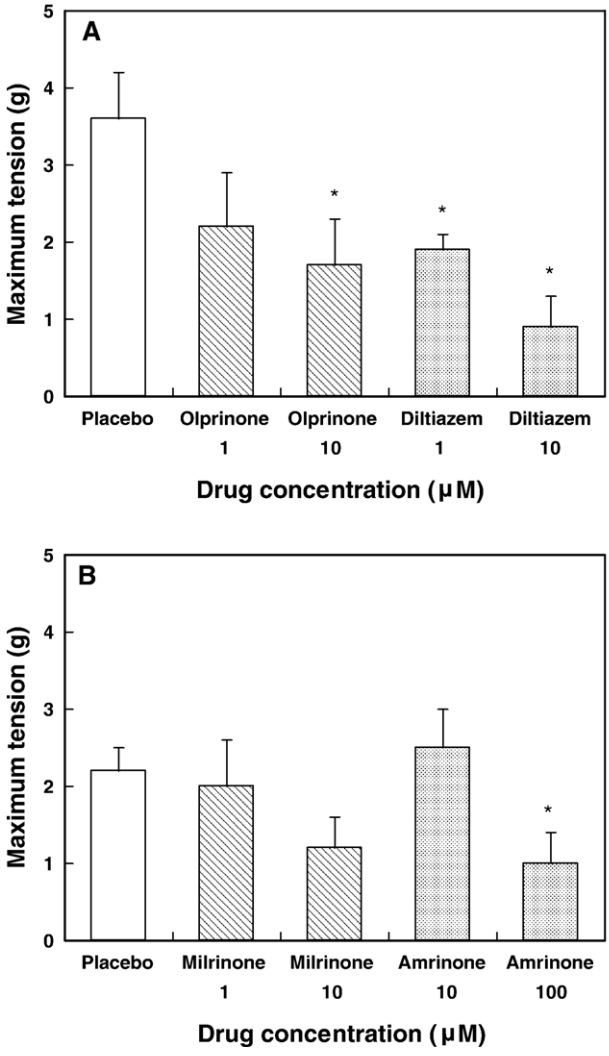

Fig. 3. (A) Effects of olprinone (1 and 10 μM) and diltiazem (1 and 10 μM) on the maximum tension of rhythmical contractile response induced by phenylephrine (100 μM) in human gastroepiploic arterial preparations without endothelium (Experiment 1). (B) Effects of milrinone (1 and 10 μM) and amrinone (10 and 100 μM) on the maximum tension of rhythmical contractile response induced by phenylephrine (100 μM) in human gastroepiploic arterial preparations without endothelium (Experiment 2). The maximum relaxation induced by 100 μM papaverine was defined as 0 g tension. All values are shown as the mean±S.E.M. determined from the results of 6 examples in all groups except the vehicle (ethanol)-treated group ($n=7$) in Experiment 2. *$P<0.05$ vs. vehicle-treated group.

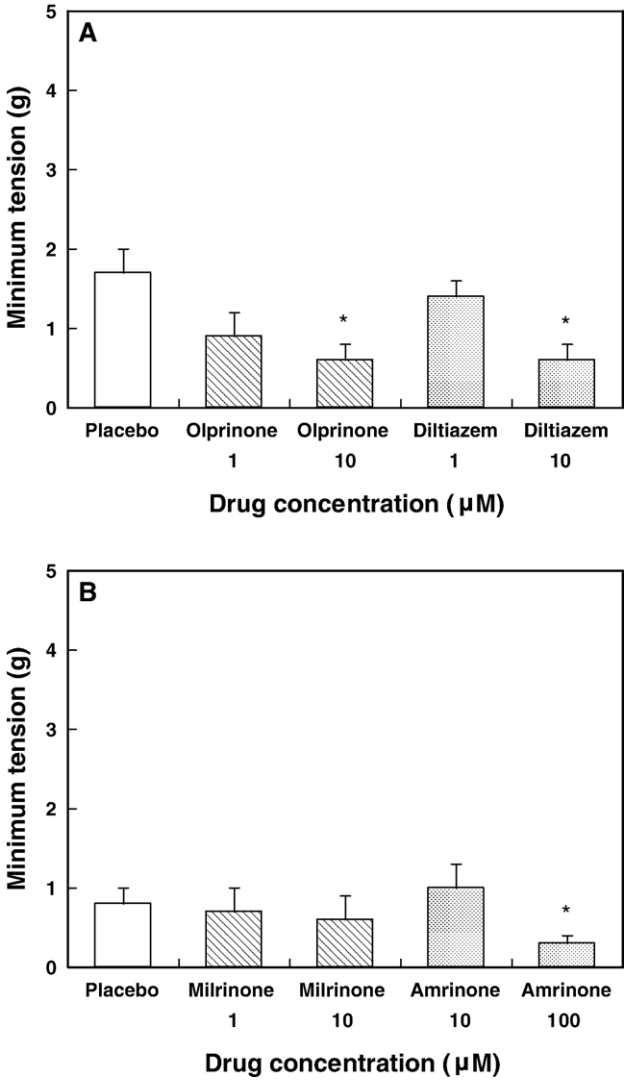

Fig. 4. (A) Effects of olprinone (1 and 10 μM) and diltiazem (1 and 10 μM) on the minimum tension of rhythmical contractile response induced by phenylephrine (100 μM) in human gastroepiploic arterial preparations without endothelium (Experiment 1). (B) Effects of milrinone (1 and 10 μM) and amrinone (10 and 100 μM) on the minimum tension of rhythmical contractile response induced by phenylephrine (100 μM) in human gastroepiploic arterial preparations without endothelium (Experiment 2). The maximum relaxation induced by 100 μM papaverine was defined as 0 g tension. All values are shown as the mean±S.E.M. determined from the results of 6 examples in all groups except the vehicle (ethanol)-treated group ($n=7$) in Experiment 2. *$P<0.05$ vs. vehicle-treated group.

4. Discussion

The major findings of the present study were that, like the calcium antagonist diltiazem, olprinone potently and remarkably inhibited the rhythmical changes of the tension induced by phenylephrine in the gastroepiploic arterial preparations, in

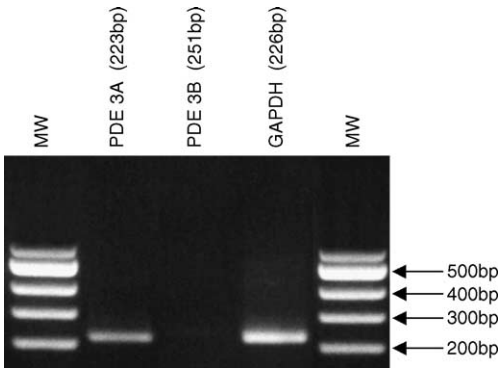

Fig. 5. Expression of phosphodiesterase (PDE) 3A in isolated human gastroepiploic artery. Total RNA isolated from dispersed cells was reversed transcribed and cDNA was amplified with specific primers for PDE3A. GAPDH, glyceraldehyde-3-phosphate dehydrogenase. No reverse transcription–polymerase chain reaction product was obtained with primers for PDE3B.

comparison with the other two PDE3 inhibitors, and that the inhibition of the PDE3A isozyme existing in an artery would be attributed to the vasorelaxant mechanism of olprinone.

cDNAs for two PDE3 isoforms, classified as PDE3A and PDE3B (Beavo, 1995), have been cloned from human and rat libraries (Meacci et al., 1992; Taira et al., 1993). PDE3A is highly expressed in cardiac muscle, vascular and visceral smooth muscle and platelets, whereas PDE3B, the product of a distinct gene, is expressed in adipocytes, hepatocytes, spermatocytes and renal collecting duct epithelium (Reinhardt et al., 1995; Liu and Mauruce, 1998; Choi et al., 2000; Palmer and Maurice, 2000). However, the existence of PDE3A and PDE3B in the human gastroepiploic artery, which was used for coronary artery bypass grafting, has not been reported yet. In the present study, we examined PDE3A and PDE3B expressions by RT-PCR in the gastroepiploic artery by using specific primers based on a conserved sequence of human primers, and have demonstrated for first time that PDE3A is expressed in the gastroepiploic artery, but PDE3B is not.

The gastroepiploic artery for coronary artery bypass grafting has a higher tendency to spasm than the internal mammary artery (Mills and Everson, 1989; Suma, 1990). He and Yang (1995) have also demonstrated that among four human arteries (gastroepiploic, internal mammary, inferior epigastric and coronary), the gastroepiploic artery is the most reactive artery in response to the four vasoconstricting substances (endothelin-1, thromboxane A_2 mimetic U46619, adrenoceptor agonist norepinephrine and depolarizing agent potassium). Phenylephrine was selected as the standard vasoconstrictor to produce spasm in vitro, because it is a full adrenoceptor agonist and induces contraction of both arteries and veins for myocardial revascularization through activation of receptor-operating calcium channels (Thorin-trescases et al., 1993; Liu et al., 1997; Adachi et al., 2000). In the present study, we observed that, unlike the tonic contraction reported in the previous studies, phenylephrine caused the spontaneously rhythmical changes of the tension being repeatedly phasic contraction as shown Fig. 1, which was blocked by a calcium channel-blocker, diltiazem, in endothelium-denuded gastroepiploic arterial preparations.

With regards to spontaneous rhythmic contraction of human large coronary arteries, Ross et al. (1980) have reported that it was dependent on external calcium, and was not observed in calcium-free solutions or with treatment of another calcium antagonist, verapamil, and further, that such phasic contractile activity was enhanced or induced by noradrenaline. In addition, Uchida (1985) has described that a rhythmic contractile response induced by 3,4-diaminopyridine could resemble clinical vasospasm in patients with variant angina and be used as an effective model to estimate the vasospasmolytic effects of various drugs. Thus, our evidence that spontaneous rhythmical changes of the tension might be produced through a mechanism related to extracellular calcium influx into smooth muscle cells blocked by diltiazem may support such previous findings, and suggests that the gastroepiploic artery could be prone to vasospasm during surgical dissection and after bypass grafting.

The correlation between contractility of the graft and long-term patency still remains unknown. Factors related to function and structure of the endothelium may also contribute (Hashimoto et al., 1991, 1992). In the present study, the artery was physically denuded of endothelium, and treated with *N*ω-nitro-L-arginine methyl ester and indomethacin in order to exclude the influence of endothelial relaxing factors (nitric oxide and prostacyclin), as practiced in previous studies (Akar et al., 1994; Adachi et al., 2000). The lower spasticity would naturally be beneficial as regards coronary bypass grafting. One fact that should be highlighted is that the higher contractility of an arterial graft, which involves a greater risk of reduced arterial diameter, and therefore decreased coronary blood flow, is disadvantageous, and that the treatment with a potent vasodilating drug, which means inhibition of perioperative spasm, is advantageous as regards short-term patency. Therefore, when it is necessary to use the gastroepiploic artery as a conduit, it may be preferable to use a vasodilator such as olprinone or diltiazem, due to their short- and long-term patency.

The other phosphodiesterase 3 inhibitor, milrinone, has been reported to be useful in preventing spasm of the internal mammary artery, which is utilized as one of the conduit vessels in human coronary bypass graft surgery (Thorin-trescases et al., 1993; Liu et al., 1997). In the present study, olprinone at a concentration of 10 μM, as well as diltiazem, significantly inhibited the frequency and maximum and minimum tensions of rhythmical changes, and its relaxant effect was endothelium-independent and direct on the smooth muscle. The inhibitory activity of olprinone was more potent than milrinone, despite the fact that olprinone acts through the inhibition of calcium mobilization mediated by the increase in cyclic AMP content of the muscle, and the IC_{50} value of olprinone for PDE3 activity was almost to the same as that of milrinone (0.63 vs. 0.76 μM, respectively, Ogawa et al., 1989). Two hypotheses exist with regard to the higher potency activity of olprinone over milrinone in in vitro arterial preparations. The first one states that olprinone inhibits the vascular contractility not only through a decrease in intracellular calcium level, but also a decrease in calcium sensitivity of contractile elements, as reported by Tajimi et al. (1991). In fact, olprinone inhibited the

verapamil-insensitive portion of the norepinephrine stimulated cytosolic calcium level and contraction, and inhibited the norepinephrine induced transient contraction but not the stimulated cytosolic calcium level in a calcium free solution (Tajimi et al., 1991). The second one states that the remarkable vasorelaxant effect of olprinone may be due to the difference in hydrophilicity and solubility in physiological solution, because olprinone and milrinone were resolved in distilled water and 100% ethanol, respectively. Therefore, olprinone may be a useful endothelium-independent vasodilator for the prevention against and treatment of spasm arising from endothelial dysfunction caused by surgical procedure or atherosclerosis.

In conclusion, olprinone causes potent and endothelium-independent relaxation of arterial spasm in the human gastroepiploic arterial preparations. These results suggest that olprinone, a potent PDE3A inhibitor, would be suitable for protecting against perioperative spasm during coronary artery bypass graft surgery.

Acknowledgments

We thank Dr. Masahiro Hiratsuka and Dr. Isao Miyashiro for isolation of the human gastroepiploic artery, and Mr. Takashi Tada and Mr. Atsushi Ichikawa for their assistance in delivering arterial preparations.

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