Interaction between endothelin and vasodilators in the human internal mammary artery

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- 1 The internal mammary artery (IMA) is the primary choice as an arterial graft for coronary artery bypass surgery. Endothelin (ET) has been recently measured with an increased release after cardiopulmonary bypass for coronary artery bypass grafting. Threshold concentrations of ET-1 have been found to amplify specifically contractions induced by noradrenaline and serotonin. This study was designed to investigate the effect of glyceryl trinitrate (GTN) and calcium antagonists on ET-1 contraction in the human IMA.
- 2 Human IMA segments taken from 21 patients undergoing IMA-coronary artery bypass grafting were mounted in an organ bath under the physiological pressure determined from their own length-tension curves. Four ring segments were allocated into four groups. One served as a control and the others were treated with GTN (10, 100 nM, or 30 µM) for 5 min or nifedipine (20 or 200 nM, or 30 µM) for 25 min before concentration-contraction curves to ET-1 were established. In separate experiments, the concentration-relaxation curves to GTN or nifedipine were established in the IMA rings precontracted with ET-1 (10 nM).
- 3 Pretreatment of IMA with GTN for 5 min did not alter the ET-1-induced contraction. Pretreatment with 20 or 200 nM of nifedipine slightly but not significantly, altered the maximum contraction induced by ET-1. Higher concentrations (30 µM) significantly reduced the maximum contraction force ($P = 0.008$). On the other hand, GTN caused $76.44 \pm 6.35\%$ relaxation in ET-1-precontracted IMA. In contrast, the nifedipine-induced relaxation was difficult to establish due to unsustained contraction to ET-1.
- 4 These results strongly suggest that in the human IMA ET-1-induced contraction may be partially inhibited by nifedipine and the mechanism of ET-1-induced contraction is only partially related to the activation of dihydropyridine-sensitive voltage dependent calcium channels in this artery. The study also demonstrates that although GTN may significantly but not completely reverse the ET-1-induced contraction it may not significantly inhibit the contraction if applied prior to contraction, possibly due to rapid tolerance. These findings may have important clinical implications in coronary artery bypass surgery.

Keywords endothelin nifedipine glyceryl trinitrate tolerance
internal mammary artery coronary bypass pharmacokinetics

Introduction

Although superior long-term patency rates of the internal mammary artery (IMA) grafting have led to the extensive use of this arterial graft [1–3], recent studies have demonstrated that blood flow through arterial grafts (in most of the patients IMA grafts were used) is inadequate for maximal exercise [4] and inadequate graft flow may cause a hypoperfusion syndrome manifested by low cardiac output, left ventricular failure, rising pulmonary wedge pressure, hypotension, and cardiac arrest [5]. Severe hypoperfusion tends to occur early in the patient's course and this situation may be worsened by high-dose-vasopressor therapy that could further reduce arterial graft flow [5]. On the other hand, the distal end of the human IMA is a site where vasospasm frequently develops due to reaction to vasoconstrictors [6–9].

Endothelin (ET), a vasoconstrictor substance derived from endothelial cells, has been suggested as the most potent vasoconstrictor in general and a potential spasmogen for blood vessels [10, 11]. In the human IMA threshold concentrations of ET-1 have been found to amplify specifically contractions induced by noradrenaline and serotonin by a calcium-dependent mechanism [12]. It has been also found that low but significant concentrations of immunoreactive ET-1 circulate in the human systemic and coronary vascular beds [13]. In particular, ET has been recently measured with a significantly increased release 20 h after cardiopulmonary bypass for coronary artery bypass grafting [14]. These findings suggest that ET may be a spasmogen for coronary bypass grafts.

Calcium antagonists provide effective therapy for patients with angina pectoris [15]. Similarly, glyceryl trinitrate (GTN) is frequently used to treat patients suffering from coronary artery disease as it dilates epicardial conductance arteries, increases collateral blood flow to ischaemic myocardium, and decreases left ventricular preload [16]. Therefore, in patients who suffer from coronary artery disease calcium antagonists such as nifedipine, and GTN have been widely applied in the clinical practice. This study was designed to examine the effects of nifedipine and GTN on ET-1-mediated contraction of the IMA.

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Methods

General

Human IMA segments were collected from 21 patients undergoing IMA graft surgery. There were 19 males and 2 females. The preoperative drug therapy in these patients was as follows: 7 patients

were on nitrates, 8 were on calcium antagonists (3 were on nifedipine and 5, diltiazem), 8 were on β -adrenoceptor blockers. Approval to use discarded IMA tissue was given by the Human Ethics Committee of the Medical City Dallas Hospital and the Institutional Review Board of the St Vincent Hospital. Following sternotomy, a full-length left IMA pedicle was carefully dissected from the chest wall. The patients were then heparinized and cardiopulmonary bypass was instituted. The left IMA was cut distally and the length for grafting to the left anterior descending artery was carefully measured and preserved. Any discarded distal IMA segments were collected and put in a container with oxygenated, physiological solution (Krebs') maintained at 4°C, and then transferred to laboratory immediately. The delay from the operating room to the laboratory was 20 min or less. The IMA was transferred into a glass dish and dissected out from its surrounding connective tissue. The IMA was then cut into 3 mm long rings, and the rings were then suspended on wires in organ baths [17]. The number of rings taken from each patient varied from 3–6. The Krebs' solution had the following composition (in mM): Na⁺ 144, K⁺ 5.9, Ca²⁺ 2.5, Mg²⁺ 1.2, Cl⁻ 128.7, HCO₃⁻ 25, SO₄²⁻ 1.2, H₂PO₄⁻ 1.2, and glucose 11. The solution was aerated with a gas mixture of 95% O₂–5% CO₂ at 37°C.

Organ-bath technique

An organ-bath technique allowing normalization of vascular rings to a physiological condition *in vitro* was used to set the vascular rings at a pressure comparable with that at the *in vivo* situation. The details of the technique were published before [18, 19]. Briefly, the rings were stretched-up in progressive steps to determine the length-tension exponential curve for each ring. A computer iterative fitting technique was used to determine the exponential line, pressure and the internal diameter. When the transmural pressure on the rings reached 100 mm Hg, determined from their own length-tension curves, the stretch-up procedure was stopped and the rings were then released to 90% of its internal circumference at 100 mm Hg. This degree of the passive tension was then maintained throughout the experiment.

The endothelium was intentionally preserved by cautiously dissecting and mounting the rings. We previously found that this technique allowed the experiments to be carried out with an intact endothelium, as determined by the functional relaxation response to acetylcholine [17]. Also, in the human isolated IMA rings we compared endothelium-dependent relaxation in the rings with intact endothelium and that in the rings in which the endothelium was removed by gently rubbing the intimal surface with a filter paper taper moistened with Krebs' solution and the results showed that the endothelium was functionally preserved in the IMA rings [18–21].

Protocol

Before and after the normalization procedure, the IMA rings were equilibrated at least for 45 min, respectively. During the equilibration period the solution in the organ bath was frequently changed. The following protocols were designed for the experiments.

Relaxation IMA rings were contracted with ET-1 (10 nM). This concentration of ET-1 was the submaximal concentration which was determined from the logistic-curve fitting equation [18, 19] from pilot experiments. Cumulative concentration-relaxation curves to GTN ($n = 7$) were then established. Only one concentration-relaxation curve was obtained from each IMA ring. From rings (taken from seven patients), a mean concentration-relaxation curve was constructed. From pilot experiments, the contraction induced by ET-1 was not sustained enough to construct concentration-relaxation curves for nifedipine because each dose of this calcium antagonist requires 25 min to reach the plateau [7, 21] whereas for GTN the time was only 3 min. Therefore, in this part of the experiments, a group of rings ($n = 4$) was contracted with ET-1 (10 nM) as a time control and another group of rings ($n = 8$) was contracted with ET-1 (10 nM), then attempted to relax with nifedipine, by being cumulatively given this vasodilator in a 25 min interval for each dose. The time for each dose (25 min) was determined from previous studies [7, 21] for nifedipine in the human IMA. The relaxation was expressed as percentage of precontraction.

Depression of contraction by pretreatment with GTN To determine whether pretreatment with GTN would alter the contraction response to ET-1, cumulative concentration-contraction curves were constructed in four rings taken from each of six patients. These four rings were equilibrated for 5 min [7, 20] with vehicle of GTN (0.1% ethanol) or 10 nM, 100 nM, or 30 μ M GTN. Those concentrations (10–100 nM) were chosen since they are similar to the optimal plasma concentration reached clinically [22, 23] and the supra-clinical concentration (30 μ M) was given to study the possible maximal inhibitory effect. A cumulative concentration-contraction curve was then constructed for ET-1. The contraction was expressed as percentage of the maximal contraction force in the control ring.

Depression of contraction by pretreatment with nifedipine To determine whether pretreatment with nifedipine would alter the contraction response to ET-1, cumulative concentration-contraction curves were constructed in four rings taken from each of eight patients for nifedipine. These four rings were equilibrated for 25 min [7] with vehicle of nifedipine (1% ethanol) or 20 nM, 200 nM, or 30 μ M nifedipine. In these concentrations, 20 and 200 nM are relevant to the optimal plasma concentrations reached clinically [24]. A cumulative concentration-contraction curve

was then constructed for ET-1. The contraction was expressed as percentage of the maximal contraction force in the control ring.

Data analysis The effective concentration of the constrictor (or dilator) agent that caused 50% of maximal contraction (or relaxation) was defined as EC_{50} . The EC_{50} was determined from each concentration-contraction (or relaxation) curve by a logistic, curve-fitting equation: $E = MA^p/(A^p + K^p)$ where E is response, M is maximal contraction (or relaxation), A is concentration, K is EC_{50} concentration, and p is the slope parameter [25].

From these fitted equations, the mean EC_{50} value and 95% confidence limit was calculated in each group. Sensitivity is expressed by EC_{50} values. Unpaired t -test or analysis of variance (and Scheffe F -test) were used to test statistical significance. $P < 0.05$ was considered as significant.

Drugs Drugs used in this study and their sources were: Endothelin-1, Peptides International, Inc., Louisville, Kentucky, GTN (Roussel Canada Inc., Montreal, Canada); nifedipine, a gift from Bayer, Germany. Nifedipine was diluted in ethanol (95%) to 1 mM, serially diluted in distilled water, and protected from light. GTN ampoule was diluted in ethanol to 10 mM and then serially diluted in distilled water and also protected from light.

Results

Resting vessel parameters [17, 18]

Seventy-eight IMA rings taken from 21 patients were used in this study. The mean internal diameter of these rings at an equivalent transmural pressure of 100 mm Hg (D100) was 1.91 ± 0.06 mm as determined from the normalization procedure. When the IMA rings were set at a resting diameter of 0.9 D100, the equivalent transmural pressure was 73.0 ± 0.9 mm Hg, and the resting force was 3.2 ± 0.1 g.

Relaxation by GTN and nifedipine in the IMA precontracted by ET-1

GTN caused $76.44 \pm 6.35\%$ relaxation in ET-1-precontracted IMA (Figure 1). The EC_{50} value was -6.79 ($-8.73 \sim -4.85$) log M. The precontraction was stable enough for testing the effect of GTN because of the fast action of GTN, which only took 3 min to reach the plateau for each dose. In contrast, in the ET-1-precontracted rings the maximal relaxation to nifedipine cannot be determined because the precontraction induced by ET-1 was not stable enough for the long period required for the cumulative dose-response curves, since the cumulative doses were given in a 25 min interval (see **Methods**) (Figure 2). Figure 3 shows an example of unsustained contraction in an IMA ring. However, although the pre-

Figure 1 Mean concentration ($-\log M$)-response (% relaxation) curves for glyceryl trinitrate (GTN) in internal mammary artery precontracted by endothelin-1 (ET, 10 nM). The symbols ● represent data averaged from seven rings (from seven patients). The symbols ○ represent ET-induced contraction as a contraction-time control ($n = 4$). Vertical error bars are 1 s.e. mean of mean values.

Figure 2 Mean concentration ($-\log M$)-response (% relaxation) curves for nifedipine in internal mammary artery precontracted by endothelin-1 (ET, 10 nM). Nifedipine was added into the organ bath in an interval of 25 min cumulatively. The symbols ● represent data averaged from eight rings (from eight patients). The symbols ○ represent ET-induced contraction as a contraction-time control ($n = 4$). Vertical error bars are 1 s.e. mean of mean values.

contraction induced by ET-1 was not stable enough to evaluate the relaxing effects of nifedipine, there was a difference between the nifedipine-treated curve and the time-control curve ($P = 0.03$, ANOVA, Figure 2).

Depression of contraction by pretreatment with GTN

Pretreatment of IMA with GTN for 5 min did not alter the ET-1-induced contraction (Figure 4). Pretreatment with 10, 100 nM or 30 μM GTN had little effect on the maximal contraction to ET-1 ($82.07 \pm 13.01\%$, $60.39 \pm 10.49\%$, and $76.11 \pm 25.89\%$ compared with the control, respectively, $P = 0.27$, ANOVA). Similarly, the pretreatment did not alter the

Figure 3 Representative trace showing unsustained force induced by endothelin-1 in a human internal mammary artery ring. The tone developed in response to endothelin-1 ($-7.5 \log M$) automatically reduced. ET, endothelin-1.

Figure 4 Mean concentration ($-\log M$)-contraction (%) curves for endothelin-1 (ET) when vehicle (25 μM 0.1% ethanol, ●), -8 (○), -7 (▲), or -4.5 (△) $\log M$ glyceryl trinitrate (GTN) were added 5 min before the ET curve started. Symbols represent data averaged from six rings (from six patients). Each patient provided four rings, one allocated to each treatment. Horizontal error bars are 1 s.e. mean of the effective concentrations causing 50% maximal response averaged from logistic, fitted curves from each ring. Contraction is expressed as percentage of the maximal contraction force of the control ring.

EC_{50} of the ET-1 induced contraction -8.36 ($-8.14 \sim -8.58$) in the control rings vs -8.14 ($-7.55 \sim -8.73$), -8.11 ($-7.89 \sim -8.33$), and -8.26 ($-8.04 \sim -8.48$) $\log M$ in the rings treated with GTN 10, 100 nM and 30 μM , respectively ($P = 0.7$).

Depression of contraction by pretreatment with nifedipine

Pretreatment of IMA with nifedipine for 25 min depressed the magnitude of ET-1 contraction (Figure 5). Pretreatment with 20 or 200 nM of nifedipine slightly but not significantly, altered the maximum contraction induced by ET-1 ($97.07 \pm 8.59\%$ or $65.3 \pm 12.46\%$). Higher concentration of nifedipine (20 μM) significantly reduced the maximum contraction force ($49.89 \pm 10.93\%$ $P = 0.008$ compared with the control, Scheffe F -test). However, EC_{50} values to ET-1 in the nifedipine-pretreated IMA rings were not significantly different among the control and the nifedipine treated-rings -8.11 ($-7.86 \sim -8.36$) in the control rings, -8.26 ($-8.12 \sim -8.40$), -7.97 ($-7.70 \sim -8.24$), and -8.03 ($-7.79 \sim -8.27$) $\log M$ in 20, 200 nM or 30 μM nifedipine treated-rings ($P = 0.47$, ANOVA).

Figure 5 Mean concentration ($-\log M$)-contraction (%) curves for endothelin-1 (ET) when vehicle ($25 \mu M$ 1% ethanol, $\bullet$), -7.7 ($\circ$), -6.7 ($\blacktriangle$), or -4.5 ($\triangle$) $\log M$ nifedipine (NIF) were added 25 min before the ET curve started. Symbols represent data averaged from eight rings (from eight patients). Each patient provided four rings, one allocated to each treatment. Horizontal error bars are 1 s.e. mean of the effective concentrations causing 50% maximal response averaged from logistic, fitted curves from each ring. Contraction is expressed as percentage of the maximal contraction force of the control ring.

Discussion

This study reveals that in the human IMA 1) inhibitory effects of the calcium antagonist nifedipine on ET-1-induced contraction is incomplete and therefore ET-1-induced contraction involves a mechanism other than the activation of dihydropyridine-sensitive voltage-dependent calcium channels; 2) GTN significantly reverses the ET-1-induced contraction in the human IMA whereas the pretreatment with GTN has little efficacy to inhibit the contraction; and 3) although ET-1-induced contraction is not sustained for a long period, it evokes a high contraction force in this artery. These findings may have important clinical implications for patients undergoing coronary artery-IMA grafting.

ET has been suggested as the most potent vasoconstrictor in general and a potential spasmogen for blood vessels [10, 11]. It has also been suggested that the sensitivity of smooth muscle to ET is both species and tissue specific [26–28] and that ET-1 may play an important role in regulating vascular tone by modulating the conversion of angiotensin I to angiotensin II [29]. At low dosages ET induces vasodilatation and at high dosages, vasoconstriction in resistance vessels of normal humans. In addition, blockade of voltage-operated calcium channels prevents ET-induced vasoconstriction and unmasks the vasodilator effect of high ET dosages [30]. It has been demonstrated that ET-1 stimulates ET_A receptors in vascular smooth muscle cells to induce contraction whereas ET_B receptors are located in endothelial cells to mediate EDRF (NO) release [31]. Taken together with other findings [12, 14], ET may be a spasmogen for coronary bypass grafts. Therefore, we designed the pre-

sent study to investigate the effect of ET-1 in the IMA and the interaction between this vasoconstrictor and vasodilator substances GTN and nifedipine, which are frequently used in patients undergoing coronary artery bypass grafting.

Previous reports on the actions of endothelin-1 *in vitro* have suggested that the vasoconstrictor effect of ET-1 depends on extracellular calcium and is inhibited by the calcium-channel blockers such as nifedipine and nicardipine [30–34]. Other studies demonstrated that calcium antagonists may effectively antagonize the ET-induced vasoconstriction *in vitro* and *in vivo* in dogs [35–37]. This effect is also observed in microvessels such as in intracerebral microarterioles [38]. ET has also been suggested to be an important regulator of the levels of intracellular calcium, through interaction with a novel site [39]. However, on the other hand, it was suggested that ET produces vasoconstriction via ultimately accelerating Ca^{2+} influx through voltage-dependent Ca^{2+} channels but that the binding site is distinct from that of dihydropyridines [40]. Other studies have demonstrated that the mechanism of voltage-dependent channel may be of only minor importance [41–44]. Removal of extracellular calcium or addition of the calcium antagonist nicardipine attenuates but does not abolish responses to ET and therefore contractile effects of endothelin cannot be explained solely by an action on voltage-dependent calcium channels [45, 46]. Instead, endothelin may activate L-type calcium channels [44, 47] and hence the entry of extracellular calcium only secondarily to the activation of non-specific cation channels [44], although a study suggested that the calcium antagonist binding sites associated with L-type calcium channels are not the primary site of action of endothelin [48]. This mechanism is only partially inhibited by selective Ca^{2+} entry blockers such as nifedipine. Furthermore, it has been proposed that the action of ET involves utilization of superficially bound calcium in addition to release of calcium from intracellular stores and that ET indirectly promotes the breakdown of phosphoinositol and the activation of protein kinase C and these mechanisms are also not affected by Ca^{2+} entry blockers [34, 46]. Therefore, ET appears to cause vasoconstriction by several mechanisms.

The present study demonstrates that in the human IMA ET-1-induced contraction is partially inhibited by the selective Ca^{2+} entry blocker nifedipine. In the present study, pretreatment with the therapeutic concentrations of nifedipine (20–200 nM) only reduced the ET-1-induced contraction to 97.07–65.3% compared with the control. However, our results disagree with previous observations that Ca^{2+} antagonists do not prevent ET-1-induced contractions [49]. In our study, at high concentration (30 μM), nifedipine significantly reduced the contraction to about 50% ($P < 0.001$, Figure 5). Using our technique (measurement of the individual diameter-passive tension exponential curve and the optimal resting point, frequent wash, and long equilibration), a stable contraction of IMA rings has been obtained for many vasoconstrictors [7] but such a contraction for ET-1 was not able

to be established. Due to unstable contraction to ET-1 in this artery, the relaxing effect of nifedipine is difficult to establish. It is possible that pretreatment with calcium antagonists and β -adrenoceptor antagonists may affect the effect of the drugs in *in vitro* experiments. However, the technique used in our study should minimize such an influence although it may not be completely excluded. In regard to relaxation, there was a difference between the nifedipine-added and the time-control IMA rings (Figure 2). However, no difference was seen regarding the maximal relaxation. These observations further demonstrate that the selective Ca^{2+} entry blocker nifedipine partially inhibits the ET-1 induced contraction. Therefore, the present study in the human IMA supports the previous studies on the mechanism of ET-1-induced contraction that this contraction is only partially through the voltage-dependent calcium channel.

GTN has been demonstrated to attenuate the vasoconstrictor effects of ET in the human fetal-placental circulation [50], coronary circulation [37], or rabbit aorta [51] although, in contrast, in human resistance vessels, blockade of voltage-operated Ca^{2+} channels but not cyclic GMP-dependent vasodilatation may be an effective tool to inhibit ET-induced vasoconstriction [30]. In addition, GTN has been shown to reverse completely the ET-induced phosphorylation of myosin light chain in coronary artery smooth muscle [52]. However, despite the fact that nitroprusside-induced relaxation in the human IMA has been studied [53], the effect of GTN on ET-1-induced contraction has not been investigated. In the present study, ET-1-induced contraction in the human IMA is reversed by GTN and the EC_{50} of the relaxation is within therapeutic range (-6.79 , -8.73 ~ -4.85 log M). Therefore, our study suggests that, in the clinical setting, GTN is an effective vasodilator for ET-1-induced contraction in the human IMA. On the other hand, the present study demonstrates that the ET-1-induced contraction is not fully reversed by GTN in the human IMA indicated by the fact that the maximal relaxation was 76.44%. In contrast, GTN has been demonstrated to reverse completely the contraction induced by U46619 [7] or α -adrenoceptor agonists [20]. This observation is partially in accordance with others [30]. Our results may suggest that the ET-1-induced contraction may be effectively but not completely reversed by cyclic GMP-dependent vasodilatation.

In the previous studies [7, 20], we have observed that although GTN is a potent vasodilator in reversing contraction induced by a number of vasoconstrictors such as the thromboxane A_2 mimetic U46619 [7] and α -adrenoceptor agonists [20], it is less effective in the human IMA if applied to prevent the contraction induced by these constrictors although the mechanism is unclear. In this study, we continuously observed this phenomenon. We have found that although the high concentration of GTN was used to incubate the IMA rings, it did not significantly reduce the contraction induced by ET-1 (Figure 4). Therefore, it is believed that this is a common

phenomenon rather than an occasional observation. We have previously proposed a hypothesis that the GTN-induced increase of cGMP may be terminated before the increase in intracellular calcium from the vasoconstrictor stimulus [7]. This proposal may be closely related to rapid tolerance to GTN. In fact, tolerance of GTN is well recognized both clinically and experimentally [54, 55]. It has been proposed that GTN-induced tolerance affects at least two major sites in the cascade of events between the initial site of GTN action and guanylate cyclase activation [55]. Both hypotheses are based on the cGMP-guanylate cyclase cascade and may be essentially similar. It was reported that 20 min incubation with GTN in organ baths made tachyphylaxis (rapid tolerance) to GTN in porcine coronary arteries [56]. Although in our experiments, GTN was added into the organ bath 5 min before the contraction curve to ET-1 was constructed, by the time the highest dose of ET-1 was given it would be more than 20 min. Rapid tolerance perhaps is the most possible explanation for the ineffectiveness of GTN in preventing the ET-1-induced contraction. Another possible explanation accounting for the ineffective prevention of ET-1-induced contraction in the human IMA is the tolerance due to preoperative or intraoperative administration of GTN. However, this is unlikely the cause in our present study because in ET-1-precontracted rings GTN induced more than 75% relaxation, despite the ineffective pretreatment. In addition, to minimize the possible effects of pretreatment of the patients with drugs during equilibration we frequently washed the rings, together with the long incubation-time should have allowed the elimination of the previous drug therapy as aforementioned [20, 21].

We realize that although the findings from this study are useful in predicting the effects of vasodilators on ET-induced contraction these *in vitro* results must be carefully applied to an *in vivo* environment.

Another interesting finding of the present study is that ET-1-induced contraction is not sustained in the human IMA. Others have reported that in rat isolated heart ET-1 causes marked and long-lasting coronary constriction and the effect is not influenced by Ca^{2+} channel blockade [57] and that ET produced a profound and long-lasting reduction in coronary blood flow with electrocardiographical evidence of myocardial ischaemia [37]. In the present study, although ET-1 induced a strong contraction (5.57 ± 0.93 g) in the human IMA, and in some ET-1-contracted rings, which were attempted to reverse the contraction by washout, even frequent washing hardly reduced the tone to the baseline, the contraction force automatically reduced with the time (Figures 2 and 3). In contrast, we have previously observed that TxA_2 [7], α -adrenoceptor agonists [19–21], and potassium [7] may induce a sustained contraction in this artery. In comparison with the findings in other vessels [58], our findings may suggest that the stability of ET-1-induced contraction is vessel-selective. In fact, a recent report [59] has suggested that tachyphylaxis to ET may be induced in rat aortic rings and may be a

possible mechanism for sustained decreases in systemic vascular resistance. It is unclear whether the unsustained contraction to ET-1 observed in the human IMA is related to tachyphylaxis.

In conclusion, the present study suggests that in the human IMA ET-1-induced contraction may be partially inhibited by nifedipine and the mechanism of ET-1-induced contraction is only partially related to the activation of dihydropyridine-sensitive voltage dependent calcium channels in this artery. The study also demonstrates that although GTN may

significantly, but not completely, reverse the ET-1-induced contraction it may not significantly inhibit the contraction if applied prior to contraction, possibly due to rapid tolerance. These findings may have important clinical implications in coronary artery bypass surgery.

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