Inhibitory effects of glyceryl trinitrate on α -adrenoceptor mediated contraction in the human internal mammary artery

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- 1 Sympathomimetic amines have been considered to be related to vasospasm. Previous studies showed that the human internal mammary artery (IMA) was capable of weak β -adrenoceptor mediated relaxation and that α -adrenoceptor agonists may induce contraction in the human IMA.
- 2 We investigated the effects of glyceryl trinitrate (GTN), a vasodilator agent often used perioperatively, on α -adrenoceptor mediated contraction in the human IMA.
- 3 Discarded human IMA segments were taken from 37 patients who underwent IMA—coronary artery bypass graft operations and equilibrated in an organ bath.
- 4 A specially designed technique was used to normalize the vessel segments under the pressure similar to the *in vivo* situation. Noradrenaline (NA), phenylephrine (PE), and methoxamine (MO) were used to contract the vessel segments.
- 5 GTN fully relaxed PE or MO (submaximal concentration) induced precontraction. Therapeutic plasma concentration of GTN relaxed 40–90% of the PE induced contraction (2.82 g , $EC_{50} = 7.92 \pm 0.06 -\log M$) and 20–90% of the MO induced contraction (1.8 g , $EC_{50} = 7.63 \pm 0.16 -\log M$). Pretreatment by the therapeutic plasma concentration of GTN inhibited the contraction induced by NA, PE in a different range. It reduced the NA induced contraction (6.9 g) by 14.8–38% ($P > 0.05$) and the PE induced contraction (4.3 g) by 7.9–39.3% ($P > 0.05$). The α_1 -adrenoceptor antagonist prazosin, at the therapeutic plasma concentration, nearly totally abolished the NA or PE induced contraction ($P < 0.001$).
- 6 We conclude that the therapeutic plasma concentration of GTN effectively relaxes the human IMA contracted by α -adrenoceptor agonists but is less effective in preventing the α -adrenoceptor mediated contraction in the human IMA and that α_1 -adrenoceptor antagonists may effectively prevent sympathomimetic amine mediated contraction in the human IMA.

Keywords internal mammary artery pharmacokinetics coronary surgery α -adrenoceptor glyceryl trinitrate

Introduction

As an important coronary artery conduit with superior long-term patency rates, internal mammary artery (IMA) has attracted the attention of many investigators in recent years. The pharmacological reactivity of the IMA relevant to clinical situation in regard to spasm (He *et al.*, 1988, 1989a,b) or patency rates possibly relating to its ability to release the endothelium-derived-relaxing-factor (EDRF) (Luscher *et al.*, 1988) of the IMA has been studied. Exogenous or endogenous sympathomimetic amines derived from ex-

ogenous or endogenous sources have been considered to be related to vasospasm (Heusch, 1990; Skarvan *et al.*, 1984; Yasue *et al.*, 1976). In large diameter arteries, α -adrenoceptors mediate contraction and β -adrenoceptors mediate relaxation. A previous study (He *et al.*, 1989b) showed that in the human IMA, β -adrenoceptor mediated relaxation was weak. Another study (He *et al.*, 1989a) indicated that the human IMA contracted strongly to α -adrenoceptor agonists so that α -adrenoceptor agonists are potential spasm producing agents

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for the human IMA. A better understanding of the mechanism of contraction and ways in which it may be prevented or released are important because peri-operative IMA spasm may compromise operative results (Sarabu *et al.*, 1987).

Glyceryl trinitrate (GTN) is often 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 (ACC/AHA, 1990). GTN is widely used in patients undergoing IMA graft surgery before, during and after operation. However, interaction between GTN and α -adrenoceptor agonists in the human IMA has not been studied yet. This study was designed to examine the effects of GTN on preventing and reversing α -adrenoceptor mediated contraction of the IMA.

Methods

General

Human IMA segments were collected from 37 patients who underwent coronary artery-IMA bypass graft surgery. The clinical characteristics of these patients are shown in Table 1. Thirty-two patients had had sublingual isosorbide dinitrate (5 mg) and transdermal GTN ointment (2.5 cm) before anaesthesia started. Approval to use discarded human IMA tissue was granted by the Human Ethics Committee of the Royal Prince Alfred Hospital, Sydney, Australia. Following median sternotomy, the left IMA was carefully dissected free from the left chest wall. The patient was then heparinized and cardiopulmonary bypass 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 theatres to the laboratory was 15 min or less. The IMA was transferred into a glass dish filled with fresh Krebs' solution aerated with 95% O₂–5% CO₂ and carefully dissected out from its surrounding connective tissue. The IMA was then cut into 3-mm-long segments, and the segments were then suspended on wires in organ baths (He *et al.*, 1989a,b). The number of rings taken from each patient

varied from 2–6. 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, SO₄^{2–} 1.2, H₂PO₄[–] 1.2, and glucose 11. The solution was aerated with a gas mixture of 95% O₂–5% CO₂ at 37 ± 0.1° C (mean ± s.e. mean). The IMA rings were allowed to be equilibrated for at least 1 h before normalization procedures started.

Organ-bath technique

A specially designed normalization technique was used in this study. Full details of this technique have been published previously (He *et al.*, 1988, 1989b). In brief, the IMA rings were stretched in progressive steps to determine the length-tension curve for each IMA ring. The internal circumference of each ring suspended with a pressure of 100 mm Hg was determined from its length-tension curve. 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 their internal circumference at 100 mm Hg. This degree of the passive tension was then maintained throughout whole experiments. After normalization, IMA rings were equilibrated at least for another 45 min. The total equilibration period before adding any drugs into organ baths is about two and a half hours. During the equilibration period the bathing solution was changed for a few times. It has been demonstrated that 90 min equilibration period is sufficient to conduct pharmacological experiments in human tissues previously exposed to drugs (Ahlner *et al.*, 1986).

Endothelium Integrity of endothelium plays an important role in vascular activity. We have previously demonstrated the functional integrity of endothelium following procedures of the organ-bath technique by the relaxing response of the endothelium to acetylcholine in canine IMA rings (He *et al.*, 1988). In normal rings (+E) precontracted with phenylephrine maximum relaxation to acetylcholine was 93.3 ± 2.4% and for intimal rubbed rings (–E), the relaxation was 3.6 ± 3.0%. In this study, we paid particular attention to carefully mount the IMA rings on the wires in the organ bath so as to minimize the damage of endothelium. Furthermore, the integrity of endothelium was tested again in this study. In the normal IMA rings acetylcholine induced 61.0 ± 14.0% relaxation of U46619 (10 nM) induced-contraction, whereas in the rings without endothelium (rubbed off mechanically (Angus *et al.*, 1986)) acetylcholine did not induce any relaxation at all.

Protocol

Relaxation α -adrenoceptor agonists phenylephrine (PE) and methoxamine (MO) were used to contract IMA rings to a stable level. The concentration of PE and MO were submaximal concentration which were determined from the logistic-curve fitting equation (He *et al.*, 1988, 1989b) from the previous work (He *et al.*, 1989a) for PE (5.0 μ M) and a pilot study for MO (100

Table 1 Clinical characteristics of 37 patients undergoing left IMA grafting

Age (years)	60.43 ± 1.42
Sex M	35
F	2
Drug therapy	
α -adrenoceptor antagonists	3
β -adrenoceptor antagonists	22
Calcium antagonists	
Verapamil	4
Nifedipine	6
Diltiazem	9
Nitrates	32

μM). Cumulative concentration-relaxation curves to GTN were then established. Only one concentration-relaxation curve was obtained from each IMA ring. From at least six rings (taken from at least six patients), a mean concentration-relaxation curve was constructed.

Depression of contraction by pretreatment with GTN To determine whether pretreatment with GTN would alter the contraction response to α -adrenoceptor agonists noradrenaline (NA, α_1 - and α_2 -adrenoceptor agonist), or PE (α_1 -adrenoceptor agonist), cumulative concentration-contraction curves were constructed in three rings taken from each of at least six patients. These three rings were equilibrated for 10 min (He *et al.*, 1989a) with vehicle of GTN (0.1% ethanol), 10 nM GTN, and 100 nM GTN. These concentrations were chosen since they are similar to optimal plasma concentration reached clinically (Wei & Reid, 1979; Yap *et al.*, 1978). A cumulative concentration-contraction curve was then constructed for NA or PE.

Depression of contraction by pretreatment with an α_1 -adrenoceptor antagonist prazosin To determine whether the contraction induced by α -adrenoceptor antagonists NA or PE would be altered by pretreatment with α_1 -adrenoceptor antagonist, prazosin, cumulative concentration-contraction curves were constructed in three rings from each of at least six patients. Two of three rings from each patient were equilibrated for 30 min with 10 nM or 100 nM prazosin for NA concentration-contraction curves. For PE concentration-contraction curves, 55 nM or 550 nM prazosin was used to pretreat the rings. These concentrations were chosen because they are similar to the plasma concentrations reached clinically. The third ring was set up as a control (without pretreatment). At least one ring from each patient was set as the control.

Clonidine-induced contraction In 10 IMA rings, concentration-contraction curves to clonidine, an α_1 - and α_2 - (Shibata *et al.*, 1989) but α_2 - preferential adrenoceptor agonist (Decker *et al.*, 1984) were constructed. In other rings, 10 nM GTN (9 rings), 100 nM GTN (7 rings), or 1 mM yohimbine, an α_2 -adrenoceptor antagonist, was added in the organ bath 10 min (for GTN) or 30 min (for yohimbine) before the concentration-contraction curve to clonidine was constructed.

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 (Nakashima *et al.*, 1982).

From this fitted equation, the mean EC_{50} value $\pm$ s.e. mean was calculated in each group. Unpaired *t*-test or

one way analysis of variance were used to test statistical significance between different constrictors and dilators.

Drugs

(-) noradrenaline bitartrate, 1-phenylephrine hydrochloride (Sigma, St Louis, Missouri); methoxamine hydrochloride (Wellcome N.Z. Limited); GTN (David Bull Laboratories, Melbourne, Australia); prazosin hydrochloride (Pfizer); clonidine (Sigma).

Results

Resting vessel parameters (He *et al.*, 1988, 1989a,b)

One hundred fifty-two IMA rings taken from 37 patients were used in this study. The mean internal diameter of these rings at an equivalent transmural pressure of 100 mm Hg (D100) was 2.31 ± 0.04 mm as determined from the normalization procedure. When the IMA rings were set at a resting diameter of 0.9D100, the equivalent transmural pressure was 75.99 ± 0.49 mm Hg, and the resting force was 4.87 ± 0.13 gram.

Relaxation by GTN in the IMA precontracted by PE or MO

GTN fully (100%) relaxed the PE or MO precontracted IMA (Figures 1 and 2). The EC_{50} value was -7.92 ± 0.06 log M in the PE precontracted IMA and -7.63 ± 0.16 log M in the MO precontracted IMA ($P > 0.05$).

Figure 1 Mean concentration ($-\log M$)—response (% relaxation) curves for glyceryl trinitrate (GTN) in internal mammary artery precontracted by $5 \mu\text{M}$ phenylephrine (PE). The symbols $\bullet$ represent data averaged from 8 rings (from 7 patients). Horizontal error bar is 1 s.e. mean of effective concentration causing 50% maximal response. The symbols $\circ$ represent PE-induced contraction as a contraction-time control. Vertical error bar is 1 s.e. mean of mean value.

Figure 2 Mean concentration ($-\log M$)—response (% relaxation) curves for glyceryl trinitrate (GTN) in internal mammary artery precontracted by $100 \mu M$ methoxamine (MO). The symbols $\bullet$ represent data averaged from 9 rings (from 6 patients). Horizontal error bar is 1 s.e. mean of effective concentration causing 50% maximal response. The symbols $\circ$ represent MO-induced contraction as a contraction-time control. Vertical error bar is 1 s.e. mean of mean value.

Figure 3 Mean concentration ($-\log M$)—contraction (force, g) curves for noradrenaline (NA) when vehicle ($20 \mu M$ 0.1% ethanol, $\bullet$), -8 ($\blacktriangle$), or -7 ($\circ$) $\log M$ glyceryl trinitrate (GTN) were added 10 min before the start of the NA curve. Symbols represent data averaged from 8 rings (from 6 patients). Each patient provided 3 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.

Depression of contraction by pretreatment with GTN

Pretreatment of IMA with GTN for 10 min slightly depressed the magnitude of NA contraction (Figure 3). The maximum contraction force was depressed by 10

Figure 4 Mean concentration ($-\log M$)—contraction (force, g) curves for phenylephrine (PE) when vehicle ($20 \mu M$ 0.1% ethanol, $\bullet$), -8 ($\blacktriangle$), or -7 ($\circ$) $\log M$ glyceryl trinitrate (GTN) were added 10 min before the start of the PE curve. Symbols represent data averaged from 7 rings (from 7 patients). Each patient provided 3 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.

nM GTN, from 6.9 g to 5.88 g ($P > 0.05$) and further depressed by 100 nM GTN to 4.28 g ($P > 0.05$). The EC_{50} values for NA were not statistically different ($P > 0.05$). Similarly, the maximum contraction by PE (4.3 g) was slightly depressed by pretreatment with 10 nM GTN to 3.96 g and further depressed to 2.61 g but these maximal contractions did not differ from one another statistically ($P > 0.05$). Again, there was no significant difference between the EC_{50} values for PE between the control rings and the rings pretreated with GTN ($P > 0.05$) (Figure 4).

Depression of contraction by pretreatment with prazosin

Pretreatment for 30 min with 10 nM α_1 -adrenoceptor antagonist prazosin depressed the maximal contraction to NA from 4.07 g to 2.46 g ($P > 0.05$) and raised EC_{50} value by 28-fold ($P < 0.01$) indicating that 10 nM prazosin rendered the rings less sensitive to NA. Prazosin (100 nM) pretreatment totally abolished the NA-induced contraction ($P < 0.01$) and the EC_{50} value could not be determined by the logistic, curve-fitting equation (Figure 5).

Similarly, pretreatment with 5.5 nM prazosin depressed the maximal contraction force induced by PE from 3.15 g in the control group to 2.05 g ($P < 0.05$) with the EC_{50} value raised 20-fold in the rings pretreated by 5.5 nM prazosin compared with the control group ($P < 0.001$). Pretreatment by 55 nM prazosin totally abolished the PE-induced contraction ($P < 0.001$). Again, the EC_{50} value for PE could not be determined by the logistic, curve-fitting equation (Figure 6).

Clonidine-induced contraction

Clonidine only induced a small contraction force in the IMA rings (Figure 7). The maximal contraction force

Figure 5 Mean concentration ($-\log M$)—contraction (force, g) curves for noradrenaline (NA) when -8 ($\circ$), or -7 (Δ) $\log M$ prazosin were added 30 min before the start of the NA curve. Symbols represent data averaged from 8 rings (from 7 patients). Each patient provided 3 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.

Figure 6 Mean concentration ($-\log M$)—contraction (force, g) curves for phenylephrine (PE) when -7.26 ($\circ$), or -6.26 (Δ) $\log M$ prazosin were added 30 min before the start of the PE curve. Symbols ($\circ$) represent data averaged from 8 rings (from 5 patients). Symbols ($\bullet$) represent data averaged from 6 rings as a control group (without prazosin pretreatment). Each patient provided at least 3 rings, one allocated to each treatment. At least one ring from each patient allocated to the control group. Horizontal error bars are 1 s.e. mean of the effective concentrations causing 50% maximal response averaged from logistic, fitted curves from each ring.

was only 0.32 g. This was only 4.6% of NA-induced contraction and 7.4% of PE-induced contraction. This small contraction was readily inhibited by GTN or α_2 -adrenoceptor antagonist yohimbine.

Discussion

From this study, some important comments may be made. 1) α_1 -adrenoceptors seem to be predominant in the human IMA; 2) GTN fully relaxes α -adrenoceptor induced contraction in the human IMA but less effective if applied before the α -adrenoceptor agonists; 3)

Figure 7 Mean concentration ($-\log M$)—contraction (force, g) curves for clonidine when vehicle (20 μ l 0.1% ethanol $\bullet$) -8 ($\circ$), or -7 (Δ) GTN, or -6 yohimbine ($\blacktriangle$) were added 10 min (for GTN) or 30 min (for yohimbine) before the start of the clonidine curve. Symbols represent data averaged from different number of rings ($\bullet$ 11 rings; $\circ$ 9 rings; $\blacktriangle$ 5 rings, and Δ 7 rings). Vertical error bars are 1 s.e. mean of maximal contraction force.

pretreatment with α_1 -adrenoceptor antagonist prazosin is very effective in inhibiting contraction induced by α -adrenoceptor agonists.

The advantages of the methodology used in this study has been fully described in the previous studies (He *et al.*, 1988, 1989b). The normalization procedure allows the IMA rings to be subjected to circumstances which are similar to those which act on the artery *in vivo*. The pressure on the IMA rings was 76 mm Hg which is very close to the clinical setting. The passive tension on the IMA rings in the organ baths was totally determined by their own length-tension curves so the experiments were conducted under a scientific basis. The diameter of the IMA in the present study was 2.3 mm, measured at a transmural pressure of 100 mm Hg. This diameter was similar to that in the previous studies (He *et al.*, 1989a,b).

In the present study, human IMA rings were contracted by NA, PE, and MO. The maximal contraction was 6.9 g by NA, an α_1 - and α_2 -adrenoceptor agonist, and 4.3 g by PE, an α_1 -adrenoceptor agonist. We tested clonidine, a mixed α_1 - and α_2 - but α_2 - preferential agonist induced contraction in the human IMA. It only induced very small contraction. It is recognized that in vascular smooth muscle the post-synaptic α_1 -adrenoceptor is the predominant vasoconstrictor type, but stimulation of post-synaptic α_2 -adrenoceptors can also induce vasoconstriction (Timmermans, 1989). Our results showed that α_1 -adrenoceptor seemed to be predominant in the human IMA since 1) α_1 -adrenoceptor agonists (PE, MO) contracted the IMA; 2) the contraction by α_1 - and α_2 -adrenoceptor agonist NA was totally abolished by the α_1 -adrenoceptor antagonist prazosin; 3) α_2 -preferential agonist clonidine only induced a very small contraction. However, to clarify further this point, a more selective α_2 -adrenoceptor agonist should be used. A detailed study on this in our laboratory using UK14304, a highly selective α_2 -adrenoceptor agonist is underway.

Relaxation by GTN in the IMA

In the present study, we found that GTN fully relaxed α -adrenoceptor agonists PE or MO contracted IMA. This is in accord with the effects of GTN on potassium or thromboxane A_2 (Tx A_2) contracted IMA (He *et al.*, 1989a). The relaxation effect was not agonist selective since EC_{50} for PE or MO was not different ($P > 0.05$). These EC_{50} values ($-7.9 \log M$ for PE and $-7.6 \log M$ for MO) were in the range of plasma concentration reached clinically and it suggested that if an IMA is contracted by α -adrenoceptor agonists excellent relaxation effects of GTN may be expected.

The contraction by mixed α_1 - and α_2 -adrenoceptor agonist NA was not stable enough to test the relaxing effects of GTN in this study and the previous study (He *et al.*, 1989a). Clonidine, an α_2 -preferential agonist, induced only very small contraction force so relaxation effects of GTN was not tested.

Depression of contraction by pretreatment with GTN or prazosin

GTN used clinically at an infusion rate of $37\text{--}175 \mu\text{g min}^{-1}$ intravenously raises the plasma level to $0.5\text{--}2.7 \text{ ng ml}^{-1}$ (8.7 to $7.9 -\log M$) (Wei & Reid, 1979; Yap *et al.*, 1978). In the present study, 10 nM GTN, a concentration easily reached clinically, and 10-fold higher concentration were added in the organ bath 10 min before the α -adrenoceptor agonists (NA, PE) were added. The results showed that the pretreatment only partially inhibits the contraction. Neither maximal contraction force nor EC_{50} values were statistically different ($P > 0.05$). Similar results were observed in the thromboxane A_2 mimetic (U46619) or potassium induced contraction in the IMA pretreated with GTN in the previous study (He *et al.*, 1989a) although the relaxing effects of GTN on potassium or thromboxane A_2 contracted human IMA were demonstrated. This study further supports that the relaxing effects of GTN in the human IMA are not constrictor-selective.

Effects of drug therapies on patients should be concerned in any study on human tissues. In this study, all the specimens were taken from patients who underwent coronary artery bypass grafting operations. These patients were on a range of medications. Obviously, it is better to study effects of drugs in the tissue which has not been subjected to these drugs or other interacted drugs before it is taken from patients as to avoid the influence by the drug therapy. We have always been trying to fulfil this principle. For instance, in a previous study on β -adrenoceptor function in the human IMA (He *et al.*, 1989b), we studied the β -adrenoceptor function in the IMA rings which had not been exposed to any β -adrenoceptor antagonists. Unfortunately, this is not always practicable. In the patients suffering from coronary artery diseases, it is rare that vasodilators, particularly nitrates are not given before surgery to relieve angina or to prevent myocardial infarction. Therefore, this study was conducted in the IMA segments which were taken from the patients subjected to drug therapies. Every effort was made to reduce or avoid the influence of previous drug therapies. For in-

stance, the equilibration period was 2.5 h and the bathing solution was changed at least 5 times during this period. Ahlner *et al.* (1986) have indicated that 90 min equilibration was sufficient for human tissue experiments to give a stable and reproducible contraction. In another study (Mikkelsen *et al.*, 1979) the equilibration period in experiments on human mesenteric arteries and veins, which was taken from patients who had had a variety of drugs including nitric oxide, was 2–3 h. Therefore, we are confident that in our experiments the influence of the previous drug therapy was minimum. However, the influence of drug therapies such as GTN tolerance on the tissue may not absolutely be excluded. Clinical studies (Nabel *et al.*, 1989; Stewart *et al.*, 1987) have shown that tolerance to nitrates may develop in patients who receive nitrate therapy. The tolerance may also develop in bovine coronary arteries in *in vitro* experiments (Henry *et al.*, 1988, 1989). In this study, effects of GTN on prevention of contraction of the human IMA to α -adrenoceptor agonists (PE or NA) were limited. A possible explanation is tolerance of the tissues to GTN because of the previous treatment. However, there are some facts that do not support this explanation. Firstly, if tolerance develops, it should also affect the relaxation of GTN on precontracted vessels. This is obviously not the case in this study because in α -adrenoceptor agonist (PE or MO) contracted IMA segments GTN induced full (100%) relaxation. With the dosage (100 nM) used for pretreatment of the IMA rings, GTN caused 90% relaxation in either MO- or PE-induced contraction (EC_{90} for GTN was $-7.0 \pm 0.1 \log M$ in MO-induced contraction and $-7.0 \pm 0.2 \log M$ in PE-induced contraction in the IMA rings). Secondly, there was no difference observed in the tissues taken from patients ($n = 32$) previously treated with nitrates and the tissues taken from patients ($n = 5$) without previous nitrate treatment in response to GTN in the present study and the experiments were randomized. Similarly, in a previous study (He *et al.*, 1989a), 38% of 53 patients did not receive nitrate treatment prior to surgery and no difference in response to GTN was observed in the IMA segments taken from these patients and in the IMA segments taken from the rest of the patients who were not on nitrates. Finally, our equilibration technique should be sufficient enough to minimize the influence of previous drug therapies as aforementioned.

Tachyphylaxis (tolerance) to GTN which was added in organ baths 10 min before the IMA rings were contracted by PE or NA may be another possible explanation for the relative ineffectiveness of GTN in preventing contraction of the IMA rings by α -adrenoceptor agonists. Sakanashi and associates (1991) reported 20 min incubation with GTN in organ baths made tachyphylaxis to GTN in porcine coronary arteries. However, it is unlikely the reason in the present study. In this study, short time of pretreatment (10 min) of the IMA rings by GTN was chosen because this was the time in the precontracted rings when the relaxation reached a plateau at each concentration of GTN (varied from 5 to 10 min) in pilot experiments as well as in a previous study (He *et al.*, 1989a). Therefore 10-min pretreatment does not seem to desensitize the rings regarding the response to GTN.

It is not clear why GTN is an excellent relaxation agent for the human IMA but is a less effective one in preventing contraction of the artery. The phenomenon occurs when the artery is contracted by either α -adrenoceptor agonists (in the present study) or other constrictors (potassium or U46619) (He *et al.*, 1989a). Therefore, this phenomenon is not constrictor-selective and must be related to the mechanism of the relaxation effects of GTN. GTN raises cyclic guanosin 5'-monophosphate (cGMP) by releasing nitric oxide in the smooth muscle cell, which stimulates the guanylate cyclase to initiate the sequestration of Ca^{2+} from cells (Ignarro *et al.*, 1985; Rapoport & Murad, 1983) so that GTN is very effective in relaxing smooth muscle cells in the presence of raised intracellular Ca^{2+} regardless of how the Ca^{2+} was raised initially. The fact that GTN is less effective in preventing contraction in the human IMA may suggest that if GTN is applied prior to raised intracellular Ca^{2+} the increase of cGMP in time may be too short to sequester the later raised Ca^{2+} from the smooth muscle cells. However, this hypothesis needs to be further examined.

In contrast, pretreatment with the α_1 -adrenoceptor antagonist prazosin (10 or 100 nM) effectively abolished the NA induced contraction. In another group, 5.5 nM prazosin, the optimal plasma concentration reached when prazosin was given orally (Wood & Bolli, 1976), significantly decreased the maximal contraction by PE with EC_{50} value raised by 28-fold ($P < 0.01$). The 10-fold higher concentration totally abolished the maximal contraction. These results indicated effective and clinically significant inhibitory effects of α_1 -adrenoceptor antagonists on α -adrenoceptor mediated contraction in the human IMA.

Vascular spasm is usually defined as a strong contraction which severely reduces or shuts down the intraluminal blood flow. The exact cause of IMA spasm perioperatively is unknown. Possible causes are direct mechanical stimulation of the smooth muscle, the local release of chemical spasmogens such as thromboxane A_2 , serotonin, noradrenaline and histamine, local changes in temperature or pH (Skarvan *et al.*, 1984). It

is difficult to detect which of the above causes is responsible for the spasm in specific situations. Therefore, effects of a wide range of vasoconstrictor or vasodilator substances in the artery should be studied to give a scientific basis for understanding the nature of spasm. In particular, sympathomimetic amines from exogenous or endogenous sources have been considered to be related to vasospasm (Heusch, 1990; Skarvan *et al.*, 1984; Yasue *et al.*, 1976). The average age of the patients undergoing IMA—coronary artery bypass grafting operations is about 60 years. Frequently, these patients need α -adrenoceptor antagonist therapy such as prazosin as a treatment for their hypertension. α -adrenoceptor agonists such as methoxamine, adrenaline and NA are often used to treat hypotension perioperatively. Therefore, the knowledge of the effects of these sympathomimetic amines on the IMA is important. The results from the present study may have important clinical implications particularly in the patients who receive sympathomimetic amine therapy. Also, it may be important to consider that perioperative IMA spasm may be caused by multiple substances acting in an additive or synergistic manner. The findings from the present study are additions to the knowledge of physiological and pharmacological characteristics of the human IMA.

In conclusion, the present study indicates that GTN may rapidly and fully release spasm suspected to be induced by sympathomimetic agents (for instance, during sympathomimetic agent administration) but it is less effective in preventing the spasm induced by sympathomimetic agents. It also shows that α_1 -adrenoceptor antagonist prazosin is effective in preventing the sympathomimetic agent-induced spasm. Although use of α_1 -adrenoceptor antagonists early post IMA-coronary artery grafting may not be recommended because of its hypotensive systemic effects, it is at least implied that if a patient is on α_1 -adrenoceptor antagonists (prazosin or other antagonists as some patients need these drugs for antihypertension purposes) the IMA is unlikely to go into spasm in response to sympathomimetic agents.

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