Inhibition of vasoconstriction by AJ-2615, a novel calcium antagonist with α_1 -adrenergic receptor blocking activity in human conduit arteries used as bypass grafts

Ming-Hui Liu,¹ Storm H. Floten¹, Qin Yang² & Guo-Wei He^{1,2}

¹Cardiovascular Research, Providence Heart Institute & Albert Starr Academic Center, Providence St Vincent Hospital, Portland, OR, USA and ²Division of Cardiothoracic Surgery, Department of Surgery, The Chinese University of Hong Kong, Hong Kong SAR, and Anhui Heart Institute, Provincial Hospital, Anhui Medical University, China

Aims Graft spasm may develop during coronary artery bypass grafting and reversal of spasm is still challenging. The purpose of this study was to investigate the *in vitro* vascular relaxant properties of AJ-2615 in human internal mammary artery (IMA).

Methods We studied 264 IMA rings taken from 65 patients undergoing coronary artery bypass grafting surgery with organ bath technique. The interaction between AJ-2615 and various vasoconstrictors was investigated in two ways.

Results AJ-2615 caused complete relaxation in methoxamine-contracted IMA rings ($100.0 \pm 0.0\%$; $n = 8$) and nearly full relaxation in potassium chloride-contracted IMA rings ($91.4 \pm 5.7\%$; $n = 8$) or noradrenaline-contracted IMA rings ($89.3 \pm 2.8\%$; $n = 8$). AJ-2615 also induced remarkable relaxation in IMA rings contracted by other vasoconstrictors. In comparison with the α_1 -adrenoceptor antagonist prazosin, AJ 2615 showed similar maximal relaxation in IMA rings contracted by methoxamine or norepinephrine. On the other hand, incubation with AJ-2615 (0.1 – $1 \mu\text{M}$) significantly inhibited all the vasoconstrictor-mediated vasoconstriction except endothelin-1 in a concentration-dependent manner.

Conclusions The results suggested that in human IMA, AJ-2615 has an inhibitory effect on vasoconstriction mediated by a variety of vasoconstrictors and the mechanism of relaxation may be related to its calcium antagonism and α_1 -adrenergic receptor blocking activity. AJ-2615 may have important clinical implications for patients undergoing coronary artery bypass surgery for reversing and preventing graft spasm.

Keywords: α_1 -adrenergic receptor, AJ-2615, calcium channel antagonist, coronary artery bypass grafting, internal mammary artery

Introduction

Calcium channel antagonists such as nifedipine, verapamil, and diltiazem were demonstrated to be useful as anti-hypertensive, antianginal, and antiarrhythmic agents in various clinical settings. We used verapamil in combination with nitroglycerin in the preparation of more spastic conduit arteries during coronary artery bypass grafting

(CABG) [1]. However, we have previously demonstrated that classical calcium antagonists are effective in inhibition of the voltage-operated channel (VOC or L-type calcium channel) but less effective to inhibit the receptor-operated channel (ROC) in the human internal mammary artery (IMA) *in vitro* [2, 3]. AJ-2615, a dihydrodibenzothiepin derivative with a structure different from that of clinically used calcium channel antagonists, was shown to possess both calcium channel antagonistic effect and α_1 -adrenergic blocking activity [4, 5]. AJ-2615 was demonstrated to have long-acting antihypertensive effects in experimental hypertensive animals [6] and to be a potential antihypertensive therapy with favourable carbohydrate metabolism and lipid-lowering activity in patients with hypertension [7].

Correspondence: Professor G.-W. He, Department of Surgery, The Chinese University of Hong Kong, Block B, 5 A, Prince of Wales Hospital, Shatin, N.T., Hong Kong SAR, China. Tel.: (852) 26450519; Fax: (852) 26451762; E-mail: gwhe@cuhk.edu.hk

Received 4 January 2001, accepted 17 May 2001.

The IMA is the graft of choice for myocardial revascularization owing to superior graft patency and increased long-term survival compared with saphenous vein grafts [8, 9]. The IMA has also been suggested to be less spastic than the radial artery [10]. Graft spasm is one of the most hazardous complications in coronary bypass surgery. The mechanism of graft spasm is still unclear and may include mechanical, physical, and pharmacological stimulation. Further, reversal of graft spasm is often challenging and the most effective therapy for the spasm is still in controversy. Nitrovasodilators, calcium antagonists, papaverine, or other vasodilators such as phosphodiesterase inhibitor or thromboxane A₂ antagonists are currently used or studied [11–14]. These vasodilators have, however, advantages and disadvantages. For example, nitrates are very effective to inhibit the ROC but have less effect in inhibition of the VOC [3]. In addition, as mentioned above, calcium antagonists are potent in inhibiting the VOC but the effect on the α -adrenoceptor-mediated contraction in the human IMA is limited [15]. Human IMA as well as radial artery has been demonstrated to have α_1 -adrenoceptors predominantly [16]. Therefore, a vasodilator possessing both calcium antagonistic and α -adrenergic receptor blocking effect is desirable. AJ-2615 possesses both calcium channel antagonistic effect and α_1 -adrenergic blocking activity and therefore it may prove of particular value in antispastic therapy in graft spasm. The present study was designed to investigate the inhibitory effects of AJ-2615 on various vasoconstrictor-mediated vasoconstriction in human IMA preparations with emphasis on both calcium channel antagonistic effect and α_1 -adrenergic receptor blocking activity of this drug *in vitro*.

Methods

Human IMA segments were collected from 65 patients undergoing coronary artery bypass surgery disregarding the preoperative drug therapy. There were 47 males and 18 females with an age of 63.1 ± 9.1 (mean $\pm$ s.d.) years. Approval to use discarded IMA tissue was given by the Institutional Review Board of St Vincent Hospital. The discarded distal IMA segments proximal to the bifurcation were collected and placed in a container with oxygenated physiological solution (Krebs) maintained at 4°C and delivered to the laboratory. All IMA preparations were tested within 6 h. 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.

Organ bath technique

The IMA was placed in a glass dish with oxygenated Krebs solution and the surrounding connective tissue was dissected out. The vessel was cut into 3 mm long rings and the number of rings taken from each patient was 4 or 8. Two hundred and sixty-four IMA rings were investigated in the present study. Artery rings were mounted on two parallel stainless steel wire hooks in a 25 ml glass organ bath containing Krebs solution, maintained at $37 \pm 0.1^\circ\text{C}$ and continuously bubbled with 95% O₂–5% CO₂. The lower wire hook was attached to a micrometer-adjustable support leg and the upper to an isometric force transducer (model FT03, Grass Instruments) to record changes in isometric force, which were amplified and recorded on a polygraph chart recorder (model 79, Grass Instruments). After a 60 min equilibration period, a normalization technique was applied to set the vascular rings at a pressure comparable to that at the *in vivo* situation. The details of this technique have been published before [17, 18]. Briefly, each arterial ring segment was stretched up in progressive steps to determine the individual length-tension curve. A computer iterative fitting program (VESTAND 2.1, Yang-Hui He, Princeton University, NJ.) was used to determine the exponential curve, the pressure, and the internal diameter. When the transmural pressure on each ring reached 100 mmHg, determined from its own length-tension curve, the stretch-up procedure was stopped and the ring was released to 90% of its internal circumference at 100 mmHg. This degree of the passive tension was then maintained throughout the experiment. After the normalization procedure, the IMA rings were equilibrated for at least 45 min. Vasoconstrictors or vasorelaxant agents were then added to study the activity of the arteries.

In our study, rings were cautiously dissected and mounted in order to preserve the endothelial function. Although in this study the rings were not individually tested for the endothelium-dependent relaxation as this may alter the response of the vessel to subsequent reactivity to vasoactive substances, we previously demonstrated that this technique allowed the experiment to be carried out with functionally intact endothelium, as determined by the relaxation response to bradykinin in the coronary artery [19] and acetylcholine in the human isolated IMA rings [13].

Protocol

The IMA rings were randomly allocated to different groups to eliminate the influence of the preoperative drug therapy.

Relaxation AJ-2615-induced relaxation was studied in IMA rings precontracted with methoxamine (MO,

0.3 mM, $n = 8$), noradrenaline (NA, 3 μM , $n = 8$), potassium chloride (K^+ , 25 mM, $n = 8$), 5-hydroxytryptamine (5-HT, 1 μM , $n = 8$), thromboxane A_2 (TXA_2) mimetic U46619 (U46619, 10 nM, $n = 8$), and endothelin-1 (ET-1, 10 nM, $n = 8$). IMA rings ($n = 8$) contracted by ET-1 (10 nM) were studied as the time control, because in a previous study [2], we found that the contraction induced by ET-1 in the human IMA was not sustained. The concentrations of these vasoconstrictors were determined from the logistic curve fitting equation and were chosen at the equal point of the effective concentration, i.e. these concentrations are equal to $\text{EC}_{50} \sim \text{EC}_{80}$ for the MO, NA, K^+ , 5-HT, U46619, and ET-1-induced contraction in the human IMA from previous studies [2, 14, 15]. Cumulative concentration-relaxation curves to AJ-2615 were then established. Cumulative concentration-relaxation curves to α_1 -adrenergic receptor antagonist prazosin in IMA rings ($n = 8$) precontracted by MO or NA were also set up. Only one concentration-relaxation curve was obtained from each IMA ring. The relaxation was expressed as percent reversal of the agonist-induced precontraction.

Depression of contraction by pretreatment with AJ-2615
After equilibration of IMA rings for at least 45 min, 100 mM K^+ was added into the organ bath and the contraction force was recorded. The ring segment was repeatedly washed with Krebs solution to restore the baseline. To determine whether pretreatment with AJ-2615 would alter the contraction response to the vasoconstrictors (MO, NA, K^+ , 5-HT, U46619, and ET-1), cumulative concentration-contraction curves were constructed in IMA rings. Four rings from one patient were arranged for respective incubation for 30 min with 0.01, 0.1, or 1 μM AJ-2615, or with vehicle as control. The time for the incubation was decided by the average time (about 25 min) to reach a plateau for each dose of AJ-2615 in the relaxation experiment from the present study. The contraction was expressed as percentage of the contraction force induced by 100 mM K^+ .

Data analysis

The sensitivity of both vasoconstrictors and vasodilators was expressed as EC_{50} , the effective concentration causing 50% of maximal contraction or relaxation. The EC_{50} was determined from each concentration-contraction (or relaxation) curve by a logistic curve-fitting equation: $E = \text{MAP}/(\text{A}^p + \text{K}^p)$, where E is response, M is maximal contraction (or relaxation), A is concentration, K is EC_{50} concentration, and p is the slope parameter. A computerized program was used for the curve fitting and EC_{50} values were determined and expressed as $\log_{10} \text{M}$ ($\log \text{M}$ in figures). Average maximal contraction, maximal relaxation, and EC_{50} were then calculated in each group ($n = 8$).

In this study, the maximal contraction or relaxation describes the 'potency' and the EC_{50} , the 'sensitivity' [3].

Statistical analysis was performed with SPSS software (SPSS, Inc, Chicago, Ill.). All values were expressed as mean $\pm$ s.e. mean. Statistical comparisons of the percentage relaxation or contraction under different treatments were performed by two-way ANOVA (general linear model) with repeated measures, followed by *post hoc* Bonferroni test to detect the individual differences. Maximal relaxation, maximal contraction, and EC_{50} values were compared by one-way ANOVA followed by *post hoc* Bonferroni test. $P < 0.05$ was considered statistically significant. n values refer to number of ring segments from separate patients.

Materials

Drugs used in this study and their sources were: potassium chloride, methoxamine, nordrenaline (norepinephrine), 5-hydroxytryptamine and prazosin (Sigma Chemical Company, St Louis, MO.); U46619 (Cayman Chemical, Ann Arbor, KY.); endothelin-1 (Peptides International, Louisville, Kentucky). Stock solutions of U46619 and ET-1 were held frozen until required. AJ-2615 is a generous gift provided by Dainippon Pharmaceutical Co. (Osaka, Japan). The chemical name and structure of AJ-2615 is: monatepil maleate (CAS 103377-41-9 (+/-)-N-(6,11-dihydrodibenzo [b,e] thiepin-11-yl)-4-(4-fluorophenyl)-1-piperazinebutanamide monomaleate, AJ-2615). AJ-2615 was dissolved in dimethylsulfoxide (DMSO) to 10 mM, serially diluted in distilled water.

Results

Resting variables of IMA rings

The internal diameter of the 264 IMA rings at an equivalent transmural pressure of 100 mmHg (D100) was 2.34 ± 0.03 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 81.3 ± 0.4 mmHg, and the resting force was 3.6 ± 0.1 g.

Relaxant effect of AJ-2615 on IMA rings contracted by MO, NA, K^+ , 5-HT, U46619, or ET-1

AJ-2615 caused complete relaxation in MO-precontracted IMA rings ($100.0 \pm 0.0\%$) and nearly full relaxation in NA-precontracted IMA rings ($89.3 \pm 2.8\%$; $n = 8$). AJ-2615 also induced remarkable relaxation in IMA rings precontracted by other vasoconstrictors. The maximal relaxation was $91.4 \pm 5.7\%$ ($n = 8$) for K^+ , $90.7 \pm 3.9\%$ ($n = 8$) for 5-HT, $75.6 \pm 3.8\%$ ($n = 8$) for U46619, and $60.9 \pm 8.9\%$ ($n = 8$) for ET-1 (normalized

by the time control). The maximal relaxation elicited by AJ-2615 in IMA rings precontracted by ET-1 was significantly less than that by MO, NA, K^+ , and 5-HT ($P = 0.001$ – 0.004 ; one-way ANOVA). In addition, it was significantly less in the rings precontracted by U46619 than that by MO ($75.6 \pm 3.8\%$ vs $100.0 \pm 0.0\%$; $P = 0.02$; 95%CI: 2.2, 46.4%; one-way ANOVA). The differences among EC_{50} values of AJ-2615 in IMA rings precontracted by all the six vasoconstrictors were not significant ($P > 0.2$, one-way ANOVA) (Table 1). Figure 1

Table 1 Maximal relaxation (%) and EC_{50} values ($-\log_{10} M$) for AJ-2615 in human internal mammary artery rings precontracted by six vasoconstrictors.

Vasoconstrictor	Maximal relaxation (%)	EC_{50} value ($-\log_{10} M$)
Methoxamine	$100.0 \pm 0.0^{*\dagger}$	7.24 ± 0.17
Noradrenaline	$89.3 \pm 2.8^*$	6.47 ± 0.21
Potassium chloride	$91.4 \pm 5.7^*$	7.06 ± 0.15
5-Hydroxytryptamine	$90.7 \pm 3.9^*$	6.49 ± 0.23
U46619	75.6 ± 3.8	6.94 ± 0.17
Endothelin-1	60.9 ± 8.9	6.87 ± 0.15

$n = 8$ in each group; $^*P < 0.05$ vs Endothelin-1 group; $^\dagger P = 0.02$ vs U46619 group, one-way ANOVA.

Figure 1 Mean concentration ($-\log_{10} M$)-relaxation (% reversal of the agonist-induced contraction) curves for AJ-2615 in the human internal mammary artery precontracted by methoxamine (MO, ●), noradrenaline (NA, ○), potassium chloride (K, ▲), 5-hydroxytryptamine (5-HT), U46619 (U, ■) and endothelin-1 (ET, □). Number of IMA rings in each group is eight (from eight patients). Values are expressed as mean $\pm$ s.e. mean. $^*P = 0.001$ or 0.008 vs MO or K group, respectively; $^\dagger P = 0.002$ vs MO group; two-way ANOVA).

depicts the AJ-2615-induced relaxation in IMA rings precontracted by the vasoconstrictors. The relaxation for MO was significantly more than that for either U46619 ($P = 0.002$; 95%CI: 4.8, 32.7%; two-way ANOVA for all concentrations) or ET-1 ($P = 0.001$; 95%CI: 12.6, 40.6%; two-way ANOVA for all concentrations). AJ-2615 also induced more relaxation in IMA rings precontracted by K^+ than that by ET-1 ($P = 0.008$; 95% CI: 2.8, 30.8%; two-way ANOVA for all concentrations). In IMA rings precontracted by ET-1, the contraction was not sustained but the difference between AJ-2615-induced relaxation and the time control was significant ($P = 0.02$; 95% CI: 1.8, 16.1%; Two-way ANOVA for all concentrations, Figure 2). Therefore, the AJ-2615-induced relaxation in this group was normalized by the time control.

Comparison of relaxant effects between AJ-2615 and prazosin

In comparison with AJ-2615 the α_1 -adrenoceptor antagonist prazosin also induced remarkable relaxation in IMA rings precontracted by MO or NA (Figure 3). Although the maximal relaxation induced by AJ-2615 or prazosin between the comparable groups was similar, the EC_{50} values of AJ-2615 were 25-fold (7.24 ± 0.17 vs 8.63 ± 0.7 $-\log_{10} M$, 95%CI: 0.68, 2.09 $\log_{10} M$, $P = 0.001$, one-way ANOVA; $n = 8$) or 280-fold (6.47 ± 0.21 vs 8.92 ± 0.13 $-\log_{10} M$, 95%CI: 1.74, 3.15 $\log M$, $P = 0.001$, one-way ANOVA; $n = 8$) higher

Figure 2 Mean concentration ($-\log_{10} M$)-relaxation (% reversal of endothelin-1-induced contraction) curves for AJ-2615 (●) in the human internal mammary artery. Number of IMA rings in each group is eight (from eight patients). Values are expressed as mean $\pm$ s.e. mean. $^*P = 0.02$ vs control group (○), two-way ANOVA).

Figure 3 Mean concentration ($-\log_{10}$ M)-relaxation [% reversal of methoxamine (a) or noradrenaline (b)-induced contraction] curves for AJ-2615 (●) in the human internal mammary artery. Number of IMA rings in each group is eight (from eight patients). Values are expressed as mean $\pm$ s.e. mean. The maximum relaxation between AJ-2615 and prazosin (○) group is not significantly different ($P > 0.2$, one-way ANOVA).

than that of prazosin in IMA rings precontracted by MO or NA, respectively.

Depression of contraction by pretreatment with AJ-2615 in IMA rings

The percentage maximal contraction, the EC_{50} values for various vasoconstrictors, and the contraction forces (g) to 100 mM potassium chloride in the human IMA rings pretreated with AJ-2615 or vehicle are listed in Table 2. There was no significant difference in the contraction induced by 100 mM potassium chloride. The mean concentration-contraction curves to these vasoconstrictors are depicted in Figure 4. In comparison with the control rings, incubation of IMA rings with AJ-2615 at the low concentration of $0.01 \mu\text{M}$ for 30 min did not either significantly depress the contraction magnitude or shift the EC_{50} values of all the vasoconstrictors ($P > 0.2$). In contrast, incubation of IMA rings with AJ-2615 for 30 min at the concentrations of 0.1 or $1 \mu\text{M}$ significantly depressed the contraction magnitude and shifted the concentration-contraction curves rightwards of all the vasoconstrictors except ET-1 (one or two-way ANOVA). In contrast, AJ-2615 at the concentrations of 0.01 , 0.1 or $1 \mu\text{M}$ did not demonstrate either significant depression effects on ET-1-induced contraction or significant shift of the EC_{50} values in comparison with the control rings.

Discussion

In this study, we have found in the human IMA, the most commonly used arterial graft for coronary artery bypass surgery, 1) that AJ-2615 has vasorelaxant effect on various vasoconstrictor-mediated vasoconstriction and the relaxant effect is more potent in α_1 -adrenoceptor agonists and

potassium chloride-induced vasoconstriction, and 2) that AJ-2615 and prazosin have similar maximal relaxation for α -adrenoceptor agonist-mediated vasoconstriction.

The IMA is widely used as a conduit for coronary artery surgery. Graft spasm sometimes occurs during CABG and this may limit graft function by reducing luminal blood flow. Graft spasm may result from endothelial injury during harvesting, which initiates the abnormal platelet-endothelium or endothelium-smooth muscle interaction [12, 19]. In addition, various vasoconstrictors have been suggested to be the spasmogens of the human arterial grafts [20]. Among those spasmogens α -adrenoceptor stimulants are important vasoconstrictors. Elevated plasma levels of catecholamines, 5-hydroxytryptamine, thromboxane, angiotensin, and endothelin have been measured during or after coronary artery bypass grafting [20, 21]. These factors, either alone or in combination, may have implications in the genesis of graft spasm mediated by different membrane receptors or ion channels.

As mentioned before, many vasodilators are currently used either systemically or topically [2, 11, 12, 22] to reverse or prevent graft spasm. However, in some cases, the above vasodilators may not provide such benefit due to the complexity of the mechanism of spasm. In this respect, AJ-2615 may offer some advantages over classical vasodilators because of its special pharmacological properties of both calcium channel antagonism and α_1 -adrenergic receptor blocking activity.

In the present study, we aimed to test the effectiveness of AJ-2615 in the reversal and prevention of arterial graft spasm *in vitro*. For this purpose, we selected most vasoconstrictors possibly mediating graft spasm during CABG. These agents produce vasoconstriction depending on Ca^{2+} influx and/or Ca^{2+} mobilization from intracellular stores by interfering with the synthesis of inositol triphosphate (IP_3) that subsequently lead to an increase in intracellular free Ca^{2+} level. Conversely, AJ-2615, by Ca^{2+} channel antagonism, prevents Ca^{2+} entry through Ca^{2+} channels or inhibits agonist-stimulated IP_3 synthesis and thus, reverses the vasoconstriction due to a decrease in intracellular free Ca^{2+} concentration [23, 24].

We also investigated the efficacy and potency of AJ-2615 to reverse the spasm mimicked by the above vasoconstrictors in IMA rings. AJ-2615 relaxed the contraction induced by all of the six vasoconstrictors to a certain extent (Figure 1). The maximal relaxation was significantly greater for the α -adrenoceptor agonists MO or NA, which may be explained by its specific inhibitory effects on α_1 -adrenergic receptor activity.

Adrenoceptors are composed of α - and β -subtypes. In large arteries α -adrenoceptors mediate contraction, whereas β -adrenoceptors, mediate relaxation. We previously studied the β -adrenoceptor function in the human IMA and showed weak relaxation of the artery [17]. The

postjunctional α -adrenoceptor is composed of α_1 - and α_2 -subtypes. We have demonstrated that in human IMA that the postjunctional α -adrenoceptors are predominantly of the α_1 -subtype and therefore the α -adrenoceptor agonist-induced contraction is mainly mediated by activation of the α_1 -adrenoceptors [16]. Since AJ-2615 has been suggested to be an α_1 -adrenoceptor antagonist [6], it is a desirable vasodilator for the α_1 -predominant graft such as IMA.

The effect of calcium antagonists on α -adrenoceptors depends on the subtype of the receptors. Some reports have demonstrated that the antagonism is more potent against contraction mediated by α_2 -adrenoceptor than

against that by α_1 -adrenoceptor agonists in the pithed rat [25], or in perfused tail arteries of spontaneous hypertensive rats [26]. Muller [27] has demonstrated that in circumflex coronary arteries nifedipine is equally potent in antagonizing responses to both α_1 - and α_2 -adrenoceptor stimulation. In contrast, others [28] have demonstrated that calcium antagonists have a greater effect on α_1 - than α_2 -adrenoceptor mediated responses in both normotensive and hypertensive rabbits. One [15] of our previous studies shows that in human IMA, the calcium antagonist nifedipine has limited inhibitory efficacy to the vasoconstriction mediated by both the pure α_1 -agonist MO and the full α -adrenoceptor agonist NA. The

Table 2 Maximal contraction (%), EC_{50} values for vasoconstrictors ($-\log_{10}$ M), and contraction forces (CF, g to 100 mM potassium chloride (K_{100})) in human internal mammary artery rings incubated with vehicle (25 μ l 0.01% DMSO, Control) or AJ-2615.

	Maximum contraction (%)	EC_{50} ($-\log_{10}$ M) [†]	K_{100} CF (g)
Methoxamine			
Control	118.2 $\pm$ 14.1	5.43 $\pm$ 0.07	1.91 $\pm$ 0.32
AJ-2615 (0.01 μ M)	98.5 $\pm$ 9.4	5.22 $\pm$ 0.16	2.43 $\pm$ 0.31
AJ-2615 (0.1 μ M)	75.2 $\pm$ 6.6*	4.43 $\pm$ 0.09*	2.92 $\pm$ 0.39
AJ-2615 (1 μ M)	70.3 $\pm$ 8.7*	4.08 $\pm$ 0.09*	2.26 $\pm$ 0.48
Noradrenaline			
Control	189.9 $\pm$ 15.6	6.31 $\pm$ 0.19	2.00 $\pm$ 0.29
AJ-2615 (0.01 μ M)	168.0 $\pm$ 18.9	6.25 $\pm$ 0.29	1.97 $\pm$ 0.30
AJ-2615 (0.1 μ M)	150.1 $\pm$ 21.9	5.73 $\pm$ 0.21	2.75 $\pm$ 0.43
AJ-2615 (1 μ M)	119.5 $\pm$ 18.9*	5.11 $\pm$ 0.17*	2.16 $\pm$ 0.57
Potassium chloride			
Control	168.9 $\pm$ 18.7	15.71 $\pm$ 0.77	2.63 $\pm$ 0.57
AJ-2615 (0.01 M)	139.1 $\pm$ 7.1	18.43 $\pm$ 1.01	2.77 $\pm$ 0.38
AJ-2615 (0.1 M)	96.0 $\pm$ 8.8*	22.84 $\pm$ 1.42*	3.54 $\pm$ 0.58
AJ-2615 (1 M)	82.4 $\pm$ 5.7*	42.35 $\pm$ 2.85*	2.63 $\pm$ 0.59
5-Hydroxytryptamine			
Control	128.9 $\pm$ 13.9	6.63 $\pm$ 0.15	1.94 $\pm$ 0.28
AJ-2615 (0.01 μ M)	115.5 $\pm$ 16.6	6.81 $\pm$ 0.17	2.45 $\pm$ 0.33
AJ-2615 (0.1 μ M)	77.6 $\pm$ 12.3	6.78 $\pm$ 0.14	3.24 $\pm$ 0.42
AJ-2615 (1 μ M)	59.0 $\pm$ 8.9*	6.57 $\pm$ 0.08	2.85 $\pm$ 0.57
U46619			
Control	298.3 $\pm$ 31.4	8.07 $\pm$ 0.08	2.28 $\pm$ 0.70
AJ-2615 (0.01 μ M)	256.2 $\pm$ 27.1	8.01 $\pm$ 0.08	2.87 $\pm$ 0.40
AJ-2615 (0.1 μ M)	221.3 $\pm$ 14.4	7.96 $\pm$ 0.07	3.10 $\pm$ 0.77
AJ-2615 (1 μ M)	205.4 $\pm$ 13.7*	7.86 $\pm$ 0.05	2.87 $\pm$ 0.63
Endothelin-1			
Control	243.8 $\pm$ 15.5	8.01 $\pm$ 0.17	1.87 $\pm$ 0.14
AJ-2615 (0.01 μ M)	232.2 $\pm$ 23.1	7.95 $\pm$ 0.28	1.85 $\pm$ 0.30
AJ-2615 (0.1 μ M)	207.1 $\pm$ 36.8	8.17 $\pm$ 0.04	2.78 $\pm$ 0.44
AJ-2615 (1 μ M)	201.6 $\pm$ 21.1	8.08 $\pm$ 0.09	1.83 $\pm$ 0.31

$n = 8$ in each group; * $P \leq 0.05$ vs control, one-way ANOVA; [†] mM for potassium chloride.

Figure 4 Mean concentration ($-\log_{10}$ M)-contraction (percentage of the contraction forces induced by 100 mM K^+) curves to methoxamine (a), noradrenaline (b), potassium chloride (c), 5-hydroxytryptamine (d), U46619 (e), and endothelin-1 (f) when vehicle (25 μ l 0.01% DMSO, control, ●), 0.01 μ M [AJ (–8), ○], 0.1 μ M [AJ (–7), ▲], or 1 μ M [AJ (–6),] AJ-2615 were added 25 min before the contraction started in the human internal mammary artery. Number of IMA rings in each group is eight (from eight patients). Values are expressed as mean $\pm$ s.e. mean. (* $P < 0.05$, ** $P < 0.01$ vs control group, two-way ANOVA).

results of our present study demonstrated that AJ-2615 significantly inhibited the vasoconstriction mediated by both MO and NA. This finding suggests that the mechanism of the inhibitory efficacy of AJ-2615 to α -adrenoceptor agonists mediated vasoconstriction in human IMA is mainly through its α_1 -adrenergic receptor blocking activity, rather than its calcium antagonism property.

Intracellular calcium increases in response to 1) membrane depolarization (i.e. K^+ solution) and Ca^{2+} influx through VOC or (2) opening of ROC and calcium release into the cytoplasm from bound intracellular calcium stores or from extracellular stores triggered by membrane receptor activation [22]. In general, the advantages of calcium antagonists are high potency to inhibit VOC, which is the primary mechanism for regulation of intracellular calcium concentration of vascular smooth muscle. In this study, we found that AJ-2615 showed more potent reversal and prevention effects on K^+ -mediated vasoconstriction in the human IMA, and it was much weaker against ROC-mediated vasoconstriction, that is, by ET or U46619 (Figures 1 and 4).

One [2] of our previous studies showed that nitroprusside, one of the most commonly used vasodilators for patients undergoing CABG, effectively reverses the vasoconstriction induced by angiotensin II, phenylephrine, and endothelin-1, but cannot significantly depress the magnitude of the endothelin-1-evoked vasoconstriction. Another of our studies [22] suggests that in the human IMA, ET-1-induced contraction may be partially inhibited by the calcium antagonist 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. Similar to nitroprusside or nifedipine, AJ-2615 also showed partial inhibition to ET-1-induced vasoconstriction in the present study (Figures 1, 2 and 4).

In conclusion, the present study suggests that in the human IMA, AJ-2615 has an inhibitory effect on vasoconstriction mediated by a variety of vasoconstrictors and the mechanism of relaxation may be related to its calcium antagonism and α_1 -adrenergic receptor blocking activity. Owing to its special pharmacological properties, AJ-2615 may have important clinical implications for patients undergoing coronary artery bypass surgery for reversing and preventing graft spasm.

The work described in this paper was fully supported by St Vincent Medical Foundation, Portland, OR, Hong Kong Research Grants Council grants (CUHK71127/01M & CUHK7240/99M). The technical assistance of the surgical team and Kay Metzger and other nurses in the Cardiac Operating Room, St Vincent Hospital is also gratefully acknowledged.

References

- 1 He GW. Verapamil plus nitroglycerin solution maximally preserves endothelial function of the radial artery: Comparison with papaverine solution. *J Thorac Cardiovasc Surg* 1998; **115**: 1321–1327.
- 2 He GW, Yang CQ. Comparison of nitroprusside and nitroglycerin in inhibition of angiotension II and other vasoconstrictor-mediated contraction in human coronary bypass conduits. *Br J Clin Pharmacol* 1997; **44**: 361–367.
- 3 He GW, Rosenfeldt FL, Buxton BF, Angus JA. Reactivity of human isolated internal mammary artery to constrictor and dilator agents. Implications for treatment of internal mammary artery spasm. *Circulation* 1989; **80**: I141–I150.
- 4 Kurokawa M, Sato F, Hatano N, Honda Y, Uno H. A new class of calcium antagonists. Synthesis and biological activity of 11-[(omega-aminoalkanoyle)-amino]-6, 6a, 7, 8, 9, 10, 10a, 11-octahydrodibenzo [b,e]-thiepin derivatives. *J Med Chem* 1991; **34**: 593–599.
- 5 Okamura H, Fujitani B, Furukawa K, et al. Pharmacologic properties of a novel Ca^{2+} entry blocker, AJ-2615, *in vitro*. *J Cardiovasc Pharmacol* 1993; **22**: 804–809.
- 6 Ikeno A, Fukuya F, Minato H, Takeyama K, Hosoki K, Karasawa T. Antihypertensive effects of AJ-2615, a new calcium antagonist with α_1 -adrenergic blocking activity in experimental hypertensive animals. *J Cardiovasc Pharmacol* 1993; **21**: 815–821.
- 7 Sasaki J, Ogihara T, Yokoyama M, et al. Comparative effects of monatepil, a novel calcium antagonist with α_1 -admergic-blocking activity, and nitrendipine on lipoprotein and carbohydrate metabolism in patients with hypertension. *Am J Hypertension* 1994; **7**: S161–S166.
- 8 Loop FD. Internal-thoracic-artery grafts: Biologically better coronary arteries. *N Engl J Med* 1996; **334**: 263–265.
- 9 Lytle BW, McElroy D, McCarthy P. Influence of arterial coronary bypass grafts on the mortality in coronary reoperations. *J Thorac Cardiovasc Surg* 1994; **107**: 675–682.
- 10 Chardigny C, Jebara VA, Acar C. Vasoreactivity of the radial artery: Comparison with the internal mammary and gastroepiploic arteries with implications for coronary artery surgery. *Circulation* 1993; **88**: 115–127.
- 11 Izzat MB, West RR, Ragoonanan C, Angelini GD. Effect of systemic vasodilators on internal mammary artery flow. *J Thorac Cardiovasc Surg* 1994; **108**: 82–85.
- 12 Sasson L, Cohen AJ, Hauptman E, Schachner A. Effects of topic vasodilators on internal mammary arteries. *Ann Thorac Surg* 1995; **59**: 494–496.
- 13 He GW, Yang CQ. Effects of thromboxane A_2 antagonist GR32191B on prostanoid and nonprostanoid receptors in the human internal mammary artery. *J Cardiovasc Pharmacol* 1995; **26**: 13–19.
- 14 He GW, Yang CQ, Gately H, et al. Potential greater than additive vasorelaxation actions of milrinone and nitroglycerin on human conduit arteries. *Br J Clin Pharmacol* 1996; **41**: 101–107.
- 15 He GW, Acuff TE, Ryan WH, et al. Inhibitory effects of calcium antagonists on α -adrenoceptor-mediated contraction in the human internal mammary artery. *Br J Clin Pharmacol* 1994; **37**: 173–179.

- 16 He GW, Shaw J, Hughes AF, *et al.* Predominant α_1 -adrenoceptor-mediated contraction in the human internal mammary artery. *J Cardiovasc Pharmacol* 1993; **21**: 256–263.
- 17 He GW, Buxton B, Rosenfeldt F, Wilson AC, Angus JA. Weak β -adrenoceptor-mediated relaxation in the human internal mammary artery. *J Thorac Cardiovasc Surg* 1989; **97**: 256–266.
- 18 Liu M-H, Jin H-K, Floten HS, *et al.* Vascular endothelial growth factor mediated endothelium-dependent relaxation is blunt in spontaneously hypertensive rat aorta. *J Pharmacol Exp Ther* 2001; **296**: 473–477.
- 19 He GW, Yang CQ, Graier WF, Yang JA. Hyperkalemia alters EDHF-mediated hyperpolarization and relaxation in coronary arteries. *Am J Physiol* 1996; **271**: H760–H767.
- 20 He GW, Yang CQ, Starr A. Overview of the nature of vasoconstriction in arterial grafts for coronary operations. *Ann Thorac Surg* 1995; **59**: 676–683.
- 21 Downing SW, Edmunds LH. Release of vasoactive substances during cardiopulmonary bypass. *Ann Thorac Surg* 1992; **54**: 1236–1243.
- 22 He GW, Yang CQ, Mack MJ, *et al.* Interaction between endothelin and vasodilators in the human internal mammary artery. *Br J Clin Pharmacol* 1994; **37**: 173–179.
- 23 Nelson MT, Quayle JM. Physiological roles and properties of potassium channels in the arterial smooth muscle. *Am J Physiol* 1995; **267**: C799–C822.
- 24 Quast U. Do the K^+ channel openers relax smooth muscle by opening K^+ channels? *Trends Pharmacol Sci* 1993; **14**: 332–337.
- 25 Jie K, van Brummelen P, Timmermans PB, Thoolen MJ, van Zwieten PA. Effects of PN 200–110, a new calcium antagonist, on α_1 - and α_2 -adrenoceptor mediated vasoconstriction elicited by selective α -adrenoceptor agonists and catecholamines on the pithed rat. *Arch Int Pharmacodyn Ther* 1985; **278**: 72–86.
- 26 Hicks PE, Tierney C, Langer SZ. Preferential antagonism by diltiazem of α_2 -adrenoceptor mediated vasoconstrictor responses in perfused tail arteries of spontaneous hypertensive rats. *Naunyn Schmiedeberg's Arch Pharmacol* 1985; **328**: 388–395.
- 27 Muller SE. Tissue specific susceptibility of α -adrenoceptor mediated vasoconstriction to nifedipine. *Naunyn Schmiedeberg's Arch Pharmacol* 1983; **324**: 64–69.
- 28 Hamilton CA, Jardine E, Reid JL. The effects of calcium antagonists on blood pressure and responses to α -adrenoceptor agonists in hypertensive rabbits. *Clin Exp Pharmacol Physiol* 1987; **14**: 77–85.