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# Coexistence of at least three distinct beta-adrenoceptors in human internal mammary artery

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The internal mammary artery (IMA) is currently the preferred conduit for myocardial revascularization. However, perioperative vasospasm and a hypoperfusion state during maximal exercise may limit its use as a bypass graft. The mechanism of spasm has not been clearly defined. Since beta-adrenoceptor activation plays a major role in vasorelaxation, the present study was carried out to investigate the beta-adrenoceptor responsiveness of human IMA smooth muscle. Isoproterenol produced a concentration-dependent relaxation in endothelium-denuded IMA segments, precontracted with phenylephrine (maximal relaxation 46.33±5.45%). Atenolol ($10^{-6}$M) and propranolol ($2\times10^{-7}$M) inhibited isoproterenol-induced relaxation. While atenolol produced partial inhibition, propranolol caused a complete inhibition in a majority of the segments and a partial inhibition in a minority. BRL 37344, a selective beta 3-adrenoceptor agonist, produced a concentration-dependent relaxation in phenylephrine-precontracted rings of endothelium-denuded IMA (maximal relaxation 40.35±4.07%). Cyanopindolol, a beta-adrenoceptor partial agonist, produced a marked relaxation (58.65±6.2%) in endothelium-denuded IMA rings, precontracted with phenylephrine. Cyanopindolol-induced relaxation was resistant to blockade by propranolol ($2\times10^{-7}$M). Spontaneous contractions of IMA rings were also observed in some cases that were inhibited by isoproterenol and BRL 37344. This observation implies the important role of beta-adrenoceptor activation in prevention of human IMA spasm.

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The results obtained in present study indicate that human IMA smooth muscle possesses an atypical β-adrenoceptor together with $\beta_1$- and $\beta_2$-adrenoceptors. Regarding the relaxation induced in IMA rings by adding BRL 37344, the possible identical entities of IMA atypical beta-adrenoceptors and beta 3-adrenoceptors are suggested.

**Keywords:** human internal mammary artery; beta-adrenoceptors; isoproterenol; BRL 37344; cyanopindolol, myocardial revascularization, the beta-adrenoceptor responsiveness, phenylephrine, propranolol, cyanopindolol

Arterial grafts are preferred conduits in coronary artery bypass grafting (CABG) surgery. This is because of their proved long-term patency compared to vein grafts [14, 16, 24]. Among the arterial grafts, human internal mammary artery has been frequently used for myocardial revascularization. However, recent studies have demonstrated that arterial grafts may cause vascular hypoperfusion due to the small diameter, the spastic characteristics or both [13, 15]. Perioperative infarction, due to the restriction of blood flow caused by IMA spasm, resistant to pretreatment with papaverin has been reported [22]. It has also been demonstrated that arterial grafts do not provide adequate blood flow for maximal exercise and hence cause a hypoperfusion syndrome [20]. The cause of IMA spasm has not been clearly defined. Since the activation of beta-adrenoceptors located on the vascular wall plays an important role in vasorelaxation, the pharmacological study of these receptors on human IMA may provide an understanding of the mechanism of spasm and suggest a rational approach for its reversal.

In present study, the responsiveness of β-adrenoceptors of human IMA smooth muscles was investigated using some selective and non-selective β-adrenoceptor agonists and antagonists. To eliminate the role of endothelial β-adrenoceptors, endothelium was removed mechanically during the whole experiments.

## Materials and Methods

### *Preparation and sampling of tissues*

Internal mammary artery samples were obtained from patients that were diagnosed with ischemic heart disease and undergoing coronary bypass surgery. The patients' clinical characteristics including drug therapy are listed in Table I. The hospital ethical committee approved the protocol. During the operation, the discarded distal end of the IMAs were obtained, placed directly into oxygenated Krebs' solution (4 °C) and transported to the laboratory in a sealed flask within 20 min.

**Table I**

*Clinical characteristics of 30 patients undergoing internal mammary artery graft surgery*

| Clinical characteristics | *n* |
|---|---|
| Age (years) | |
| 57 ± 7.66 | 30 |
| Sex | |
| Male | 25 |
| Female | 5 |
| Drug therapy | |
| Nitrovasodilators | 30 |
| β-Adrenoceptor antagonists | 29 |
| Calcium channel blockers | 19 |
| ACE inhibitors | 8 |
| Digoxin | 4 |
| Diuretics | 2 |

*Organ bath studies*

In the laboratory isolated blood vessels were carefully cleared from surrounding tissues and cut into rings of 3 mm length. The endothelium was removed mechanically by gentle rubbing of the intimal surface of the segments with a metal rod for 30–50 seconds. Rings were placed over two parallel wires and suspended in organ bath containing Krebs' solution of the following composition (mM): NaCl 118, KCl 4.8, $CaCl_2$ 2.5, $KH_2PO_4$ 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, glucose 10. The buffer solution was gassed with a mixture of 95% $O_2$ and 5% $CO_2$ and maintained at 37 °C. The rings were stretched to a tension of 2.5–4 g, which was determined to be optimal for maximal agonist-induced tension development in preliminary experiments, and allowed to equilibrate for 2.5 hours. Tissues were washed at 15-min intervals. At the beginning of the experiments, the absence of functional endothelium was assessed by the inability of ACh (1 μM) to elicit a relaxant response. Isometric contractions were recorded on a Beckman polygraph. To obtain the relaxant responses, IMA rings were contracted submaximally with phenylephrine (10 μM). Ascending concentrations of agonists (i.e. isoproterenol and BRL 37344, a non-selective β-adrenoceptor and a selective $\beta_3$-adrenoceptor agonist, respectively) were then added cumulatively to the organ bath. Also, a dose response curve for an β-adrenoceptor partial agonist (cyanopindolol) was obtained in phenylephrine (50 mM) -precontracted rings. When antagonists (i.e. propranolol and atenolol, a non-selective and a $\beta_1$-selective adrenoceptor antagonist,

respectively) were used, the tissues were allowed to equilibrate with each compound for 30 min before the agonist concentration-response curve was repeated. In each individual preparation only one pair of agonist/antagonist was tested. To assess the relaxing ability of IMA smooth muscle, a nitrovasodilator (i.e. isosorbide dinitrate) was used at the end of experiments.

*Drugs*

Drugs used in this investigation included: acetylcholine bromide, DL-isoproterenol hydrochloride, DL-propranolol hydrochloride, phenylephrine hydrochloride (all from Sigma Chemical Co, St. Louis, MO), atenolol (ALPS Pharmac. Ind. Co. Ltd.), BRL 37344 (RR+SS)(4-[2-[2-(3-chlorophenyl)-2-hydroxyethyl] phenoxyl] acetic acid (Tocris Cooksen Ltd., UK), (-)-cyanopindolol hemifumerate (ANAWA TRADING SA, Switzerland) and isosorbide dinitrate was a gift from Arya Co. Ltd.

*Data analysis*

Agonist potency was presented as $pEC_{50}$ value (–log $EC_{50}$). Results are expressed as mean ± s.e. of *n* number of experiments. Mean values are compared by Student's *t*-test and differences between mean values were accepted as significant when $p < 0.05$.

## Results

*Relaxant effects of β-adrenergic agonists*

Phenylephrine ($10^{-5}$M) produced a submaximal contraction in endothelium-denuded rings of human IMA. Isoproterenol ($10^{-8}$–$10^{-4}$M) and BRL 37344 ($10^{-6}$–$10^{-4}$M) caused a concentration-dependent relaxation in these ring segments (Fig. 1). The $pEC_{50}$ values for isoproterenol and BRL 37344 are shown in Table II. Isoproterenol- and BRL 37344-induced maximal responses were 46.33±5.45% and 40.35±4.07% of initial tension, respectively. Spontaneous contractions were observed in some of the IMA rings which could be inhibited by high concentrations of isoproterenol and BRL 37344 (Fig. 2).

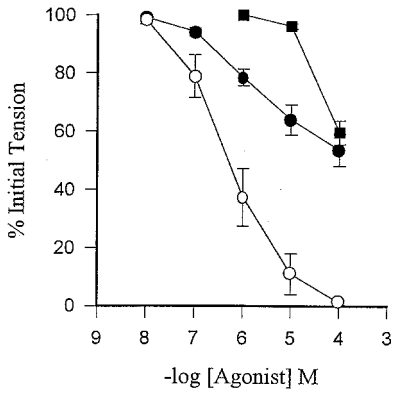


Fig. 1. Concentration-response (% initial tension) curves for isoproterenol (●, $n = 13$), BRL 37344 (■, $n = 6$) and isosorbide dinitrate (○, $n = 5$) in endothelium-denuded ring segments of human internal mammary artery precontracted with phenylephrine ($10^{-5}$M). Each point represents mean ±S.E.

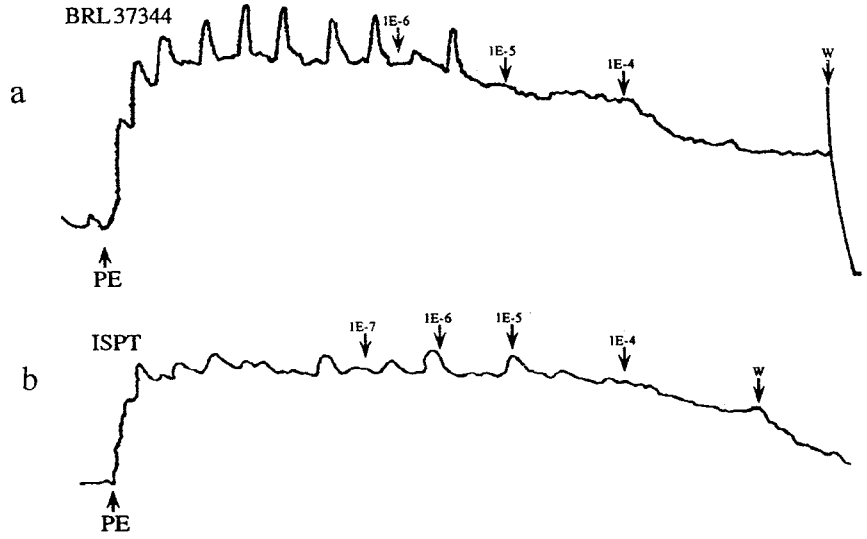


Fig. 2. Spontaneous contractions of endothelium-denuded human internal mammary artery segments, precontracted with phenylephrine (PE). At ↓ the drug was added to the organ bath using the indicated concentration. a) Suppression of spontaneous contractions by BRL 37344. b) Suppression of spontaneous contractions by isoproterenol (ISPT)

Isosorbide dinitrate induced a near-maximal relaxation (98.8±0.75% of initial tension) in endothelium-denuded IMA rings precontracted with phenylephrine ($10^{-5}$M), see Fig. 1. The $pEC_{50}$ value of isosorbide dinitrate is shown in Table II.

**Table II**

*Relaxant potencies ($pEC_{50}$ values) of β-adrenoceptor agonists and a nitrovasodilator in endothelium-denuded human internal mammary artery precontracted with phenylephrine (Mean + S.E.)*

| Drugs | $pEC_{50}$ values | Maximal relaxation (%) |
|---|---|---|
| β-Agonists: | | |
| Isoproterenol | 5.89 ± 0.15 | 46.33 ± 5.45 |
| BRL 37344 | 4.56 ± 0.02 | 40.35 ± 4.07 |
| Cyanopindolol | 4.31 ± 0.03 | 58.65 ± 6.2 |
| Nitrovasodilator: | | |
| Isosorbide dinitrate | 6.24 ± 0.25 | 98.8 ± 0.75 |

*Blockade of agonist-elicited relaxation by β-adrenergic antagonists*

Atenolol ($10^{-6}$M) and propranolol ($2\times10^{-7}$M) inhibited isoproterenol-induced relaxation. While atenolol-induced inhibition was partial (Fig. 3), propranolol-induced inhibition was complete in a majority of the segments (Fig. 4a) and partial in a minority (Fig. 4b) (See also Table III.).

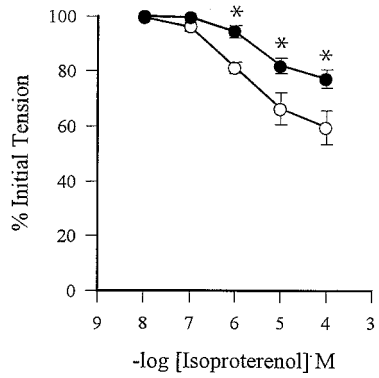


Fig. 3. Concentration-response (% initial tension) curves for isoproterenol in the absence (❍) and presence (●) of atenolol ($10^{-6}$M) in endothelium-denuded ring segments of human internal mammary artery precontracted with phenylephrine ($10^{-5}$M). Each point represents mean ±S.E. of 6 experiments (*$p<0.05$)

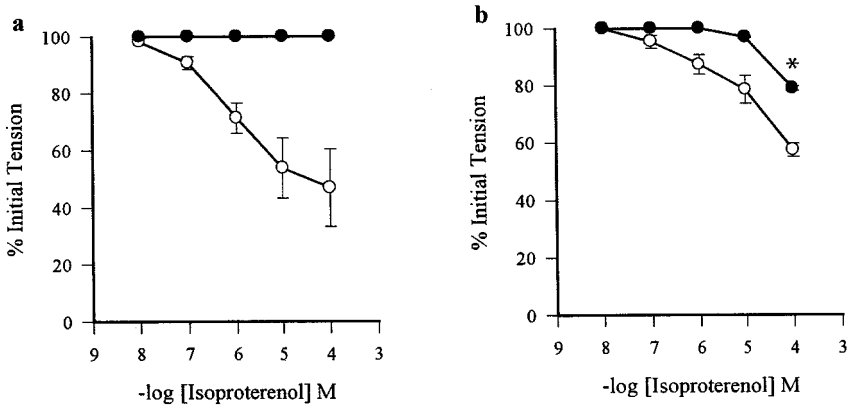


Fig. 4. Concentration-response (% initial tension) curves for isoproterenol in the absence (○) and presence (●) of propranolol ($2\times10^{-7}$M) in endothelium-denuded rings of human internal mammary artery precontracted with phenylephrine ($10^{-5}$M). a) Complete inhibition of isoproterenol-induced relaxation by propranolol. Each point represents mean ±S.E. of 5 experiments. b) Partial inhibition of isoproterenol-induced relaxation by propranolol. Each point represents mean ±S.E. of 3 experiments (*$p<0.05$)

*Effect of non-conventional partial agonist*

(-)-Cyanopindolol ($5\times10^{-6}$–$10^{-4}$M) caused a concentration-dependent relaxation of phenylephrine ($5\times10^{-5}$M) -precontracted rings of human IMA in the absence of endothelium (Fig. 5). The $pEC_{50}$ value of (-)-cyanopindolol is shown in Table II. The maximal relaxation produced by (-)-cyanopindolol was 58.65±6.2% of initial tension. (-)-Cyanopindolol-induced vasodilation was resistant to blockade by propranolol ($2\times10^{-7}$M).

**Table III**

*Isoproterenol potencies ($pEC_{50}$ values) in endothelium-denuded human internal mammary artery on the basis of the extent of inhibition by propranolol ($2\times10^{-7}$M)*

| Inhibition by propranolol | n | $pEC_{50}$ values for isoproterenol |
|---|---|---|
| Complete | 5 | 6.2 ± 0.08 |
| Partial | 3 | 5.2 ± 0.33 |

The difference between the $pEC_{50}$ values is significant ($p<0.01$)

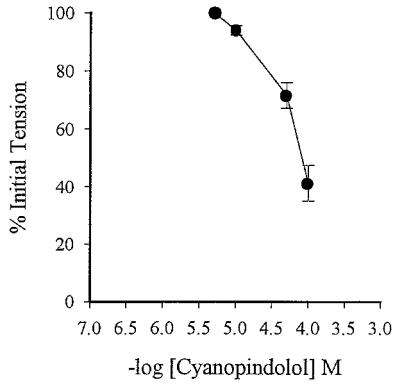


Fig. 5. Concentration-response (% initial tension) curve for cyanopindolol in endothelium-denuded ring segments of human internal mammary artery precontracted with phenylephrine ($5\times10^{-5}$M). Each point represents mean ±S.E. of 4 experiments

## Discussion

The internal mammary artery has been the conduit of choice in CABG surgery. However, perioperative spasm and inadequate blood flow for maximal exercise leading to a hypoperfusion syndrome are frequently reported using this arterial graft. The cause of vasoconstriction is still unknown. Regarding the important role of β-adrenoceptor activation in vascular relaxation, the identification of these receptors in human IMA may provide an understanding of the management of vasospasm. Functional studies have shown that $\beta_2$-adrenoceptor stimulation in endothelium-denuded human IMA rings causes vasorelaxation; in addition, this vessel possesses a relatively minor $\beta_1$-adrenoceptor component [7]. However, receptor autoradiography, using [$^{125}$I]-CYP to label $\beta_1$- and $\beta_2$-adrenoceptors, has demonstrated a high density of $\beta_2$-adrenoceptors localized to the endothelium of human IMA and fewer $\beta_2$-adrenoceptors on the smooth muscle [24]. The same report has indicated that the relaxation of internal mammary artery to (-)-isoproterenol is mediated via $\beta_2$-adrenoceptors located on the smooth muscle. Endothelial $\beta_2$-adrenoceptors, although present on the IMA, mediate other functions [24].

In order to reduce or avoid the influence of previous drug therapies in present study, the tissues were equilibrated in bathing solution for at least 2.5 h and washed every 15 minutes. Ahlner et al. [1] have shown that 90 minutes equilibration was sufficient for human tissue experiments to give stable and reproducible contractions. Therefore, we were nearly confident that the influence of the previous drug therapy was minimal in our experiments.

The results obtained in present study showed that isoproterenol induced a concentration-dependent relaxation in endothelium-denuded IMA rings precontracted with phenylephrine ($10^{-5}$M) (maximal response 46.33±5.45%; Table II). Meanwhile, the near maximal relaxing ability (over 98%) of IMA smooth muscle was assessed using isosorbide dinitrate. Regarding the extent of maximal relaxation (80.52±10.5%) for isoproterenol in endothelium-denuded IMA rings precontracted with phenylephrine, observed in another report [24], there may be an ethnic difference in this response.

Atenolol ($10^{-6}$M) and propranolol ($2\times10^{-7}$M) inhibited isoproterenol-induced relaxation of phenylephrine-precontracted IMA rings in the absence of endothelium. While atenolol-induced inhibition was partial, propranolol-induced inhibition was complete in a majority ($n = 5$) of the rings and partial in a minority ($n = 3$). This concentration of propranolol, i.e. $2\times10^{-7}$M, has been shown to block $\beta_1$- and $\beta_2$-adrenoceptors, but does not affect atypical β-adrenoceptors significantly [17]. There are reports concerning partial [12] and complete [24] inhibition of isoproterenol-induced relaxation of human IMA by propranolol, $10^{-7}$M and $5\times10^{-7}$M respectively. It should be noted that in the two groups of IMA ring segments that responded in different manner to propranolol, $pEC_{50}$ values for isoproterenol were also significantly different (Table III). No obvious correlation between $pEC_{50}$ values and patients' characteristics (data not shown) were observed which might be due to the small sample size in our experiments. In addition to the patients' characteristics, interindividual variations (e.g. receptor affinity for agonist and antagonist, differences in receptor density, etc.) may be involved in the differences observed in responding to propranolol-induced inhibition. The manner of inhibition by propranolol and atenolol for isoproterenol-induced relaxation indicates the involvement of both $\beta_1$- and $\beta_2$-adrenoceptors in IMA vasodilatation that is consistent with the previous report [7].

Cyanopindolol, a non-conventional partial agonist, is a high affinity blocker of both $\beta_1$- and $\beta_2$-adrenoceptors [17]. It also exerts a stimulant effect through $\beta_3$-adrenoceptors in rat colon [17] and through an atypical cardiostimulant β-adrenoceptor in rat and human atrium [17, 26]. In present study cyanopindolol behaved as a full agonist producing greater maximal relaxation (58.65±6.2%), compared to that induced by isoproterenol (46.33±5.45%), in endothelium-denuded IMA ring segments precontracted with phenylephrine ($5\times10^{-5}$M). The relaxant response to cyanopindolol was not antagonized by propranolol ($2\times10^{-7}$M). This points to the interaction of cyanopindolol with an atypical β-adrenoceptor in human IMA. The relaxation was slow in onset and took a much longer time, compared to isoproterenol, to attain a peak response.

The selective $\beta_3$-adrenoceptor agonist BRL 37344 is a potent stimulant of lipolysis in both brown and white adipose tissues of the rat [2, 29] and a relaxant of smooth muscles of rodent GI tract [5, 6, 8, 18, 21, 25]. Results obtained in the present

study showed that BRL 37344 induced a concentration-dependent relaxation in endothelium-denuded IMA segments precontracted with phenylephrine ($10^{-5}$M). The low potency of BRL 37344 obtained in this study (Table II) may be due to the lower sensitivity of human $\beta_3$-adrenoceptors to this agonist [19].

To accept the involvement of $\beta_3$-adrenoceptors in a physiological system the following criteria are used [3, 17, 23]: 1) the receptor should be selectively stimulated by $\beta_3$-adrenoceptor selective agonists; 2) the receptor should be stimulated by non-conventional partial agonists; 3) the receptor should be resistant to blockade by antagonists possessing only high affinity for $\beta_1$- and $\beta_2$-adrenoceptors; 4) the receptor should be blocked by $\beta_3$-adrenoceptor-selective antagonists.

For agonist-induced relaxation of human IMA, the criteria (1) and (2) were fulfilled. Regarding the third criterion, propranolol ($2\times10^{-7}$M) did not inhibit cyanopindolol-induced relaxation. Concerning the inhibition of isoproterenol-induced relaxation by propranolol ($2\times10^{-7}$M), a point should be noted. Although isoproterenol can activate $\beta_3$-adrenoceptors present in mammalian adipocytes [10, 11], previous studies have clearly demonstrated that much higher concentrations of catecholamines, compared to the concentrations that activate $\beta_1$- and $\beta_2$-adrenoceptors, are needed for activation of $\beta_3$-adrenoceptors [9, 10, 11]. In this study, isoproterenol-induced relaxation was inhibited completely, in a majority of ring segments, by propranolol ($2\times10^{-7}$M). This implies that the concentrations of isoproterenol, which were used in the experiments, had the ability to stimulate only $\beta_1$- and $\beta_2$-adrenoceptors on human IMA. It is consistent with the results obtained in previous report [4] concerning the effect of isoproterenol on canine cutaneous vessels. As it mentioned above, the partial inhibition of propranolol in a minority of segments might be related to some interindividual differences.

Some of the IMA samples showed spontaneous contractions that were inhibited by isoproterenol and BRL 37344. It may imply the relative important role of endogenous catecholamines in prevention of spasm and, therefore, blockade of β-adrenoceptors by β-adrenoceptor antagonists may exacerbate the spasm in this arterial graft.

## Conclusion

The results obtained in this study indicate that $\beta_1$- and $\beta_2$-adrenoceptors are present on human IMA smooth muscle. Moreover, the existence of an atypical β-adrenoceptor is also suggested on the basis of non-conventional partial agonist-induced relaxation.

It has been shown that atypical β-adrenoceptors in different tissues are not identical [25]. Previous studies on vascular tissues have demonstrated the existence of an atypical β-adrenoceptor distinct from $\beta_3$-adrenoceptors on rat aortic smooth muscle [27] and $\beta_3$-adrenoceptors on canine cutaneous and fat vascular smooth muscle [4, 28]. As it was mentioned above cyanopindolol has been shown to be capable of stimulating atypical β-adrenoceptors [17, 25, 26], including $\beta_3$-adrenoceptors in rat colon [17]. Concerning the relaxation produced by cyanopindolol and a selective $\beta_3$ agonist, BRL 37344, it is possible that the atypical β-adrenoceptors of human IMA smooth muscle and $\beta_3$-adrenoceptors have the same entity. This should be clarified by using a selective $\beta_3$ antagonist in further studies.

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