# Functional $\alpha_1$-adrenergic receptor subtypes in human right gastroepiploic artery[1]

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## ABSTRACT

**AIM:** To study the functional $\alpha_1$-adrenergic receptor ($\alpha_1$-AR) subtypes in human right gastroepiploic artery (RGA). **METHODS:** The effects of $\alpha_2$-AR, $\alpha_1$-AR, and $\alpha_1$-AR subtype selective antagonists on norepinephrine (NE)-induced vasoconstriction in isolated human RGA were observed by contractile function experiment. **RESULTS:** Cumulative concentration-response curves for NE were competitively antagonized in RGA by $\alpha_2$-AR selective antagonist yohimbine (p$A_2$ 6.82±0.28, slope 1.12±0.40), $\alpha_1$-AR selective antagonist prazosin (p$A_2$ 9.77±0.22, slope 0.90±0.22), $\alpha_{1A}$-AR selective antagonists RS17053 (p$A_2$ 8.42±0.20, slope 0.93±0.20) and 5-MU (p$A_2$ 8.42±0.22, slope 0.88±0.18), $\alpha_{1D}$-AR selective antagonist BMY7378 (p$A_2$ 6.84±0.32, slope 1.05±0.17), and $\alpha_{1A}$-, $\alpha_{1B}$-AR selective antagonist WB4101 (p$A_2$ 8.88±0.20, slope 1.15±0.16). The correlation coefficients between these p$A_2$ values of $\alpha_1$-AR selective antagonists with p$K_i$ values of which obtained from $\alpha_{1A}$-, $\alpha_{1B}$- and $\alpha_{1D}$-AR cloned cells are 0.95, 0.82, and 0.42. After the vessels were pretreated by chlorethylclonidine (CEC), an $\alpha_{1B}$- and $\alpha_{1D}$-AR irreversible alkylating agent, the p$D_2$ values were changed from 5.9±0.5 to 5.6±0.6 and the maximal contraction was changed from (8.9±3.2) g to (8.0±3.2) g, respectively. The difference was not significant. **CONCLUSION:** In human RGA, the contraction response is mainly mediated by $\alpha_1$-AR, of which $\alpha_{1A}$-AR plays an important role, whereas $\alpha_{1B}$- and $\alpha_{1D}$-AR are not involved in the contraction response.

## INTRODUCTION

$\alpha_1$-Adrenergic receptor ($\alpha_1$-AR) is an important mediator, which contributes to the regulation of vascular activity, and is involved in almost all-vascular smooth muscles to cause contraction response induced by norepinephrine (NE). $\alpha_1$-AR is subdivided into three subtypes ($\alpha_{1A}$, $\alpha_{1B}$, and $\alpha_{1D}$-AR) by pharmacological techniques with subtype-selective antagonists and molecular biological techniques. The corresponding cDNA coding for these receptors had been cloned by means of molecular biological methods[1,2]. The distribution of $\alpha_1$-AR and $\alpha_1$-AR subtypes has been extensively characterized in rat and rabbit vessels using functional studies and molecular biological methods[3-5]. However, very little is known regarding the distribution of $\alpha_1$-AR in human vessels. Right gastroepiploic artery (RGA) is an alternate conduit used in coronary artery bypass graft (CABG) because of its similarity to internal mammary artery (IMA). The RGA is reported to exhibit better contractility to NE than the IMA, but comparable en-

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dothelium-dependent relaxation[6]. However, it is unknown which subtype of $\alpha_1$-AR mediates NE-induced contraction. In this study the functional role of $\alpha_1$-AR and its 3 subtypes in human RGA was investigated.

## MATERIALS AND METHODS

Norepinephrine, yohimbine, propranolol, prazosin, desmethylimipramine, normetanephrine, and acetylcholine were purchased from Sigma. 8-(2-(4-(2-Methoxyphenyl)-1-piperazinyl)-8-azaspiro (4,5) decane-7-dionedihydrochloride) (BMY7378), 2-(2,6-dimethoxyphenoxyethyl) aminomethyl-1, 4-benzo-dioxane (WB4101), 5-methyl-urapidil (5-MU), and chlorethylclonidine (CEC) were purchased from Research Biochemicals. *N*-(2 -(2-cyclopropylmethoxy) ethyl) 5-choro-α,α-dimethyl-1*H*-indole-3-thylamine (RS17053) was provided by Roche Bioscience.

**Organ bath experiments** Human undistended RGA from patients (mean age, 61 a±3 a; 14 male and 2 female; range 40 to 74 years old) were obtained during gastrectomy, respectively. The vessels were immediately placed in Krebs' solution of the following composition (mmol/L): NaCl 120, $NaHCO_3$ 20, KCl 5.45, $NaH_2PO_4$1.2, $MgCl_2$ 1.2, $CaCl_2$ 2.5, glucose 10, and disodium edetic acid 0.04 (saturated with 95 % $O_2$ and 5 % $CO_2$). The vessels were then dissected free of connective tissue, cut into 2-mm ring segments for *in vitro* contractile studies. They were carefully denuded of endothelium by inserting a small forceps into the lumen and gently rolling the ring backwards and forwards in the dissecting chamber. The lack of functional endothelium was confirmed by the absence of endothelium-dependent relaxation of acetylcholine (10 mmol/L) in raised tone preparations induced by NE.

Desmethylimipramine 0.1 μmol/L, normetanephrine 1 μmol/L (to block neuronal and extraneuronal uptake of NE, respectively) and propranolol 10 μmol/L (to block β-adrenoceptors) were included in the incubation solution (yohimbine 0.1 μmol/L was added into the incubation solution when needed). The preparations were mounted at 37 °C in 10-mL organ baths containing Krebs' solution saturated with 95 % $O_2$ and 5 % $CO_2$, and attached to force displacement transducer. The preparations were equilibrated at an optimal resting tension of 3.0 g for 1 h, and primed twice with 10 μmol/L NE. After thorough washing, NE-cumulative concentration-response curve (CRC) was generated, followed by another 30-min washing. RGA were then incubated in turn with increasing amounts of yohimbine, prazosin, WB4101, 5-MU, RS17053, and BMY7378 for 40 min, after that 3 NE-CRC were generated in the presence of the above antagonists, $EC_{50}$ values and 95 % confidence limits were calculated for all CRC. The p$A_2$ values were calculated by Schild plot. RGA were incubated with CEC 50 μmol/L for 30 min; after 40-min washing (the solution without CEC), p$D_2$ values of NE and maximal contraction were determined.

**Statistics** Data were presented as mean±SD. Statistical analysis was performed by Student' *t* test.

## RESULTS

**Effects of a-AR selective antagonists on blood vessel contraction induced with NE** In RGA NE caused the vasoconstriction in a concentration-dependent manner. Cumulative concentration-contractile response curves for NE (NE-CRC) were obtained with p$D_2$ value (5.9±0.5) and the maximal contraction (8.9 g±3.2 g). Prazosin (1, 3, and 10 nmol/L) and yohimbine (0.1, 0.3, and 1 μmol/L) competitively inhibited NE-induced contraction in a concentration-dependent manner. The p$A_2$ values and slopes for antagonists were shown in Tab 1 and Fig 1.

**Tab 1. The p$A_2$ values and slopes of a-AR selective antagonists inhibiting NE-induced contraction in RGA. Mean±SD. [a]*P*>0.05 *vs* 1.**

| Antagonists | *n* | p$A_2$ | Slope |
|---|---|---|---|
| Prazosin | 5 | 9.77±0.22 | 0.90±0.22[a] |
| Yohimbine | 4 | 6.82±0.28 | 1.12±0.40[a] |

**Effects of $a_1$-AR subtype selective antagonists on blood vessel contraction induced with NE** WB4101 (3, 10, and 30 nmol/L), 5-MU (30, 100, and 300 nmol/L), RS17053 (10, 30, and 100 nmol/L) and BMY7378 (0.3, 1, and 3 μmol/L) also competitively inhibited NE-induced contraction in a concentration-dependent manner in RGA. The p$A_2$ values and slopes for antagonists were shown in Tab 2 and Fig 1.

**Effect of CEC on NE-CRC** Before and after CEC 50 mmol/L pretreatment, the p$D_2$ values (5.9±0.5 *vs* 5.6±0.6, $n$=4) and the maximal contraction (8.9 g± 3.2 g *vs* 8.0 g±3.2 g, $n$=4) of NE-CRC had no change ($P$>0.05, Fig 2).

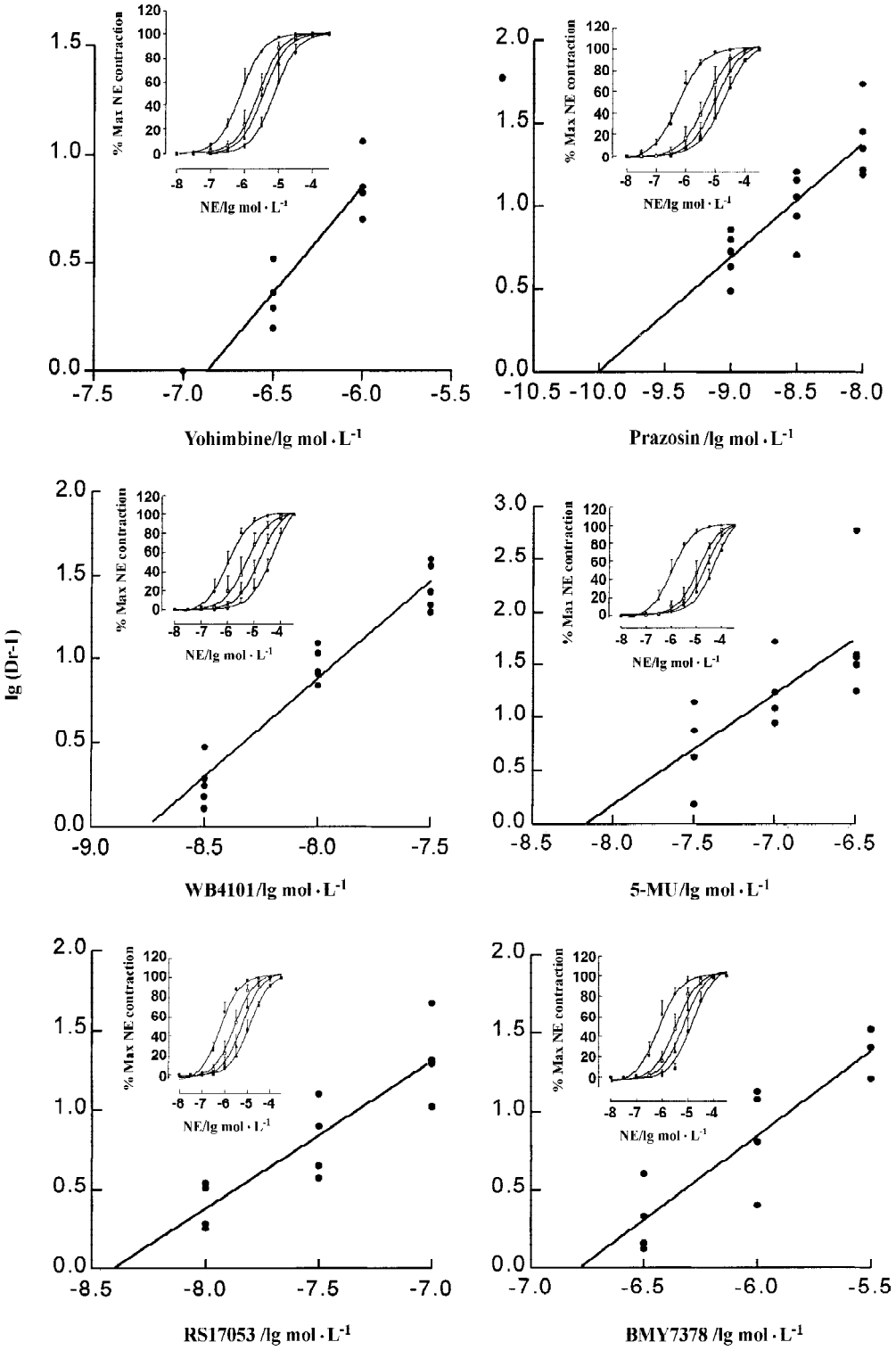


**Fig 1. Concentration-contractile response curves and Schild plot for antagonism of norepinephrine-induced contraction by a-AR and $a_1$-AR subtype selective antagonists in human RGA. Yohimbine (0.1, 0.3, and 1 mmol/L); prazosin (1, 3, and 10 nmol/L); WB4101 (3, 10, and 30 nmol/L); 5-MU (30, 100, and 300 nmol/L); RS17053 (10, 30, and 100 nmol/L); BMY7378 (0.3, 1, and 3 mmol/L).**

**Tab 2. p$A_2$ values and slopes of a$_1$-AR selective antagonists inhibiting NE-induced contraction in RGA. Mean±SD. [a]*P*>0.05 *vs* 1.**

| Antagonists | *n* | p$A_2$ | Slope |
|---|---|---|---|
| WB4101 | 5 | 8.88±0.20 | 1.15±0.16[a] |
| 5-MU | 5 | 8.42±0.22 | 0.88±0.18[a] |
| RS17053 | 4 | 8.42±0.20 | 0.93±0.20[a] |
| BMY7378 | 6 | 6.84±0.22 | 1.05±0.17[a] |

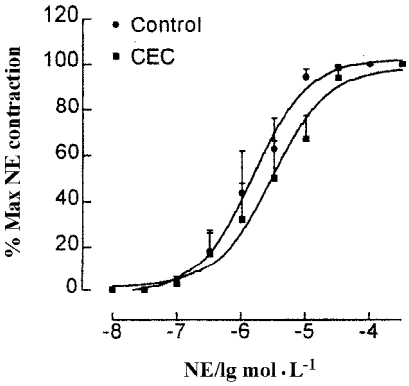


**Fig 2. Concentration-contractile response curve for antagonism of norepinephrine-induced contraction by CEC 50 mmol/L in human RGA.**

## DISCUSSION

The use of arterial grafts instead of vein grafts in CABG has been demonstrated through the years to improve survival and to reduce the recurrence of myocardial ischemia and the occurrence of late myocardial infarction. The current trend in CABG is toward complete arterial revascularization. RGA is one of the arteries used for CABG with better clinical results[7,8], because it is related to the better contraction and dilation functions[9]. The good endothelial function of the RGA might be important for graft function and patency, whereas the enhanced contractility may facilitate vasospasm, especially in the presence of high circulating level of catecholamines. Therefore it is important to investigate the primary functional $\alpha_1$-AR subtype, which contributes to NE-induced vessel contraction. In the present studies, we used $\alpha_1$-AR and $\alpha_1$-AR subtype selective antagonists as tools to assess the function of $\alpha_1$-AR subtypes, which contribute to NE-induced contraction in RGA.

In functional experiments, $\alpha_1$-AR selective antagonist prazosin antagonized NE-induced contraction with higher affinity than that of $\alpha_2$-AR selective antagonist yohimbine (p$A_2$ values were 9.77±0.22 *vs* 6.82±0.28). Because the slopes of prazosin and yohimbine were not significantly different from unity, it indicated that there was one site binding of α-AR in RGA and the contraction response was mainly mediated by $\alpha_1$-AR.

Further more, we studied the effect of $\alpha_1$-AR subtype selective antagonist on the NE-induced contraction response of RGA. $\alpha_{1A}$-AR subtype selective antagonists, including 5-MU and RS17053, antagonized NE-induced contraction with higher affinity (p$A_2$ values were 8.42 respectively), whereas BMY7378, an $\alpha_{1D}$-AR subtype selective antagonist, antagonized NE-induced contraction with lower affinity (p$A_2$ values was 6.84). In addition, the slopes for these drugs in Schild plot were not significantly different from unity. It indicates that $\alpha_{1A}$-AR might be the primary subtype, which contributes to NE-induced contraction. The further correlation analysis was performed. The p$A_i$ values for $\alpha_1$-AR subtype selective antagonists obtained from functional study were correlated with their p$K_i$ values measured in radioligand binding competition assays with membranes from HEK293 cells expressing $\alpha_{1A}$, $\alpha_{1B}$, and $\alpha_{1D}$-AR in our previous study[10]. The values for the correlation coefficient *r* were 0.95, 0.82, and 0.42. And the best correlation is with $\alpha_{1A}$-AR ($P$<0.05). It confirms that $\alpha_{1A}$-AR is a main subtype, which contributes to NE-induced contraction. Because there has no $\alpha_{1B}$-AR subtype selective antagonist now, we used CEC, an $\alpha_{1B}$ and $\alpha_{1D}$-AR irreversible alkylating agent in functional study. Before and after RGA were pretreated by CEC, the p$D_2$ values and the maximal contractile response for NE were unchanged. These results indicated strongly that in human RGA, NE-induced contraction was mainly mediated by $\alpha_{1A}$-AR subtype.

In conclusion, $\alpha_{1A}$-AR mainly contributes to the NE-induced contraction in human RGA. This result implies that $\alpha_{1A}$-AR antagonist can be used as an antispastic drug when RGA is employed for CABG.

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