Functional $\alpha_1$-adrenergic receptor subtypes in human right gastroepiploic artery

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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 ($pA_2$ 6.82±0.28, slope 1.12±0.40), $\alpha_1$-AR selective antagonist prazosin ($pA_2$ 9.77±0.22, slope 0.90±0.22), $\alpha_{1A}$-AR selective antagonists RS17053 ($pA_2$ 8.42±0.20, slope 0.93±0.20) and 5-MU ($pA_2$ 8.42±0.22, slope 0.88±0.18), $\alpha_{1D}$-AR selective antagonist BMY7378 ($pA_2$ 6.84±0.32, slope 1.05±0.17), and $\alpha_{1A}$-, $\alpha_{1B}$-AR selective antagonist WB4101 ($pA_2$ 8.88±0.20, slope 1.15±0.16). The correlation coefficients between these $pA_2$ values of $\alpha_1$-AR selective antagonists with $pK_i$ 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 $pD_2$ 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 sub-

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dothelium-dependent relaxation. However, it is unknown which subtype of α₁-AR mediates NE-induced contraction. In this study the functional role of α₁-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-dionedi hydrochloride) (BMY7378), 2-(2,6-dimethoxyphenoxyethyl) aminomethyl-1,4-benzo-dioxane (WB4101), 5-methyl-urapidil (5-MU), and chlorothelylonidene (CEC) were purchased from Research Biochemicals. N-(2-(2-cyclopropylmethoxy)ethyl) 5-choro-α,α-dimethyl-1H-indole-3-thylamine (RS17053) was provided by Roche Bioscience.

**Organ bath experiments** Human undistended RGA from patients (mean age, 61 ±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, NaHCO₃ 20, KCl 5.45, NaH₂PO₄ 1.2, MgCl₂ 1.2, CaCl₂ 2.5, glucose 10, and disodium edetic acid 0.04 (saturated with 95 % O₂ and 5 % CO₂). 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 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, EC₅₀ values and 95 % confidence limits were calculated for all CRC. The pA₂ 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), pD₂ values of NE and maximal contraction were determined.

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

**RESULTS**

Effects of α₁-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 pD₂ value (5.9±0.5) and the maximal contraction (8.9 ±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 pA₂ values and slopes for antagonists were shown in Tab 1 and Fig 1.

**Tab 1.** The pA₂ values and slopes of α₁-AR selective antagonists inhibiting NE-induced contraction in RGA. Mean±SD. *P>0.05 vs 1.

<table>
<thead>
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<th>Antagonists</th>
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<td>Prazosin</td>
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<td>Yohimbine</td>
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<td>6.82±0.28</td>
<td>1.12±0.40</td>
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Effects of α₁-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 pA₂ 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 pD₂ 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 $\alpha$-AR and $\alpha_1$-AR subtype selective antagonists in human RGA. Yohimbine (0.1, 0.3, and 1 $\mu$mol/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 $\mu$mol/L).
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 α₁-AR subtype, which contributes to NE-induced vessel contraction. In the present studies, we used α₁-AR and α₁-AR subtype selective antagonists as tools to assess the function of α₁-AR subtypes, which contribute to NE-induced contraction in RGA.

In functional experiments, α₁-AR selective antagonist prazosin antagonized NE-induced contraction with higher affinity than that of α₁-AR selective antagonist yohimbine (pA₂ 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 α₁-AR in RGA and the contraction response was mainly mediated by α₁-AR.

Further more, we studied the effect of α₁-AR subtype selective antagonist on the NE-induced contraction response of RGA. α₁A-AR subtype selective antagonists, including 5-MU and RS17053, antagonized NE-induced contraction with higher affinity (pA₂ values were 8.42 respectively), whereas BMY7378, an α₁D-AR subtype selective antagonist, antagonized NE-induced contraction with lower affinity (pA₂ values was 6.84). In addition, the slopes for these drugs in Schild plot were not significantly different from unity. It indicates that α₁A-AR might be the primary subtype, which contributes to NE-induced contraction. The further correlation analysis was performed. The pA₂ values for α₁-AR subtype selective antagonists obtained from functional study were correlated with their pKᵢ values measured in radioligand binding competition assays with membranes from HEK293 cells expressing α₁A, α₁B, and α₁D-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 α₁A-AR (P<0.05). It confirms that α₁A-AR is a main subtype, which contributes to NE-induced contraction. Because there has no α₁B-AR subtype selective antagonist now, we used CEC, an α₁B and α₁D-AR irreversible alkylating agent in functional study. Before and after RGA were pretreated by CEC, the pD₂ 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 α₁A-AR subtype.

In conclusion, α₁A-AR mainly contributes to the NE-induced contraction in human RGA. This result implies that α₁A-AR antagonist can be used as an antispastic drug when RGA is employed for CABG.

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