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European Journal of Pharmacology 413 (2001) 263–269

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Postjunctional α_{2C}-adrenoceptor contractility in human saphenous vein

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Received 10 August 2000; received in revised form 11 December 2000; accepted 19 January 2001

Abstract

The postjunctional α_2-adrenoceptor-mediated contractility was characterized in human saphenous vein derived from coronary artery bypass graft surgery. Human saphenous vein contracted to α_2-adrenoceptor selective agonists BHT-920 (5,6,7,8-Tetrahydro-6-(2-propenyl)-4*H*-thiazolo[4,5-d]azepin-2-amine dihydrochloride; $pD_2 = 6.7 \pm 0.1$) and UK 14,304 (5-Bromo-6-(2-imidazolin-2-ylamino)quinoxaline; $pD_2 = 7.2 \pm 0.1$). BHT-920-induced contractions were inhibited by the α_2-adrenoceptor antagonist yohimbine (17-Hydroxy-yohimban-16-carboxylic acid methyl ester hydrochloride; $pA_2 = 8.7 \pm 0.5$), but not by the α_1-adrenoceptor antagonist prazosin (1-[4-Amino-6,7-dimethoxy-2-quinazolinyl]-4-[2-furanylcarbonyl]-piperazine hydrochloride; 300 nM). In contrast, prazosin ($pK_b = 7.9 \pm 0.2$) potently antagonized contractions elicited by the α_1-adrenoceptor agonist phenylephrine ((*R*)-3-Hydroxy-α-[(methylamino)methyl] benzenemethanol hydrochloride; $pD_2 = 4.9 \pm 0.1$), indicating that both α_2- and α_1-adrenoceptor evoke human saphenous vein contractions. Functional antagonist activity estimates (pA_2 or pK_b) obtained for the α-adrenoceptor antagonists ARC 239 (2-[2-(4-(2-Methoxyphenyl)piperazin-1-yl)ethyl]-4,4-dimethyl-1,3-(2*H*,4*H*)-isoquinolindione dihydrochloride), WB 4101 (2-(2,6-Dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane hydrochloride) and HV 723 (α-ethyl-3,4,5-trimethoxy-α-(3-((2-(2-methoxyphenoxy) ethyl)amino)propyl)benzeneacetonitrile) against BHT-920-induced human saphenous vein contractions were 7.0 ± 0.6, 8.3 ± 0.6 and 7.7 ± 0.3, respectively. The α_2-adrenoceptor subtype affinities (pK_i) obtained in recombinant human α_{2A}-, α_{2B}- and α_{2C}-adrenoceptor competition binding assays were 8.6, 8.3 and 8.6 for yohimbine; 6.3, 8.4 and 7.0 for ARC 239; 8.4, 7.5 and 8.4 for WB 4101 and 7.5, 7.4 and 7.9 for HV 723, respectively. Taken together, the binding and functional antagonist activity estimates obtained in these investigations indicate that α_{2C}-adrenoceptor is the predominant postjunctional α_2-adrenoceptor subtype in human saphenous vein.

Keywords: Human saphenous vein; α_{2C}-adrenoceptor

1. Introduction

The α_2-adrenoceptor has been shown to exhibit subtype heterogeneity that may serve as the basis for development of drugs with improved therapeutic and safety profiles (Bylund et al., 1995; Smith et al., 1997). To date, three human α_2-adrenoceptor subtypes (α_{2A}, α_{2B} and α_{2C}) have been classified using functional and molecular techniques (Bylund et al., 1995). Clonidine, apraclonidine, guanfacine and guanabenz are examples of α_2-adrenoceptor type-selective but subtype non-selective drugs. These agents are used therapeutically to lower blood pressure, reduce intraocular pressure and for aiding withdrawal from narcotics, alcohol and tobacco addictions (Hoffman and Lefkowitz, 1996).

The α_2-adrenoceptor subtypes exhibit distinct distributions in the central nervous system (CNS) and the periphery (Nicholas et al., 1996; Smith et al., 1997). Thus, the development of α_2-adrenoceptor subtype-selective agents may allow more precisely targeted therapy compared to current nonspecific α_2-adrenoceptor drugs.

The physiological role of the α_{2C}-adrenoceptor subtype in autonomic cardiovascular function has been defined with the use of the recently developed α_{2C}-adrenoceptor subtype specific knockout mice (MacDonald et al., 1997). Findings from cardiovascular studies using α_{2A}-, α_{2B}- and α_{2C}-adrenoceptor knockout mice indicate that, while the peripheral α_{2A}- and α_{2B}-adrenoceptor modulate blood

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0014-2999/01/$ - see front matter © 2001 Published by Elsevier Science B.V.
PII: S0014-2999(01)00772-5

pressure, the α_{2C}-adrenoceptor does not play a measurable role in the control of systemic blood pressure (MacDonald et al., 1997). This finding is consistent with the notion that an α_{2C}-adrenoceptor selective drug has a minimized potential for systemic blood pressure effects. The development of α_{2C}-adrenoceptor subtype-selective agonists and antagonists will help to further elucidate the physiological role(s) of this receptor and may provide leads for a variety of therapeutic areas. Towards this end, a functional human α_{2C}-adrenoceptor bioassay would be valuable in the development of and functional characterization of subtype-selective agonists and antagonists.

Human saphenous vein is readily available from coronary artery bypass graft surgery (Docherty, 1987). Recently, in human saphenous vein derived from varicose vein surgery, the postjunctional α-adrenoceptor contractility has been shown to be mediated predominantly by an α_2-adrenoceptor (Smith et al., 1992; Steen et al., 1984). Moreover, studies by Gavin et al. (1997) indicate this α_2-adrenoceptor response is largely mediated by the α_{2C}-adrenoceptor subtype. The present investigations evaluate the potential of human saphenous vein from coronary artery bypass graft surgery to serve as an α_{2C}-adrenoceptor bioassay. To this end, the postjunctional α-adrenoceptor-mediated contractility of human saphenous vein was studied using the selective α_2-adrenoceptor agonists BHT-920 (Macia et al., 1984) and UK 14,304 (De Ponti et al., 1996) and the α_1-adrenoceptor agonist phenylephrine (De Ponti et al., 1996). The α_2-adrenoceptor antagonist activity of yohimbine (De Ponti et al., 1996), ARC 239 (Parsley et al., 1999), WB 4101 (Parsley et al., 1999) and HV 723 (Muramatsu et al., 1990) was determined against BHT-920 in human saphenous vein. Affinity estimates for these α-adrenoceptor antagonists at the recombinant human α_{2A}-, α_{2B}- and α_{2C}-adrenoceptor were obtained in binding assays using cells expressing single receptor subtypes. The resulting functional human saphenous vein potencies and recombinant human α_2-adrenoceptor subtype binding affinities for the four antagonists were subjected to correlation analysis to further characterize the α_2-adrenoceptor contractile responses in human saphenous vein.

2. Methods

2.1. Human saphenous vein

Human saphenous vein was obtained from coronary artery bypass graft surgery patients with a known age range of 49 to 83 years. Human saphenous vein from 36 predominantly (> 75%) male patients were procured by Morristown Memorial Hospital (MMH, Morristown, NJ, USA) and the Hackensack University Medical Center Institute for Biomedical Research (HUMC, Hackensack, NJ, USA). MMH human saphenous vein were stored and shipped at 4°C in Roswell Park Memorial Institute (RPMI) solution with 25 mM HEPES buffer, glutamax I, minimal essential media (MEM) vitamin and nonessential amino acid solutions, antibiotic/antimycologic solutions and 10,000 U/ml heparin (Gibco, Grand Island, NY). HUMC human saphenous vein were stored and shipped at 4°C in heparinized autologous blood. MMH human saphenous vein was received at the Schering-Plough Research Institute (SPRI) 24–72 h post removal. HUMC human saphenous vein was received within 24 h of removal. On the day of arrival, human saphenous vein was either used fresh or cryopreserved for use at a later date.

2.2. Cryopreservation of human saphenous vein

Cryopreservation methods used have been previously described in Valentine et al. (1999). Cryopreservation preserves functional neuromuscular activity in a variety of blood vessels (Ellis and Müller-Schweinitzer, 1991; Valentine et al., 1999). Briefly, human saphenous vein ring segments 5 mm long and 3–7 mm in diameter were placed into Nunc cryovials q.s. to 1.8 ml with chilled, heat inactivated fetal bovine serum containing 1.8 M dimethyl sulfoxide (DMSO) and 0.1 M sucrose. The vials were placed in a Nalgene cryofreezing container equilibrated at 4°C for 10 min and then transferred to −80°C overnight for controlled freezing. The cryovials were then removed from the cryofreezing container and stored at −80°C until use. Cryopreserved human saphenous vein was thawed within 2.5 min using a dry bath heating unit. The vessel rings were immediately transferred to chilled RPMI solution.

2.3. Functional α-adrenoceptor bioassay in human saphenous vein

Stainless steel tissue hooks and 2-0 silk were used to anchor the human saphenous vein ring segments in 25.0 ml organ baths (Q-bath, Radnoti Glass Technology, Monrovia, CA) and attach them to Grass FT-03 force transducers (Astro-Med, West Warick, RI). Isometric tension was continuously recorded using a model K2G physiograph (Astro-Med). The organ baths were filled with a pH 7.4 Krebs-style buffer (118 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 24.9 mM $NaHCO_3$, 11.1 mM glucose, 2.55 mM $CaCl_2$) maintained at 37°C and continuously aerated with 95% O_2–5% CO_2 gas. Rings were placed under 1.0 g initial resting tension and equilibrated for 2 to 3 h. Tissues were tested for responsiveness with norepinephrine (100 μM) and washed during the equilibration period. During the experimental portions of the procedure, contractions to rising cumulative concentrations of an agonist were observed in the absence or presence of an antagonist. Antagonist equilibration time was 1 h before the agonist challenge. Upon completion of the experiment, a KCl (80 mM)-induced contraction was performed.

2.4. Ligand binding studies

Chinese hamster ovary (CHO)-K1 cells stably expressing the recombinant human α_{2A}-, α_{2B}- and α_{2C}-adrenoceptor were grown in complete Ham's F12 media containing 10% fetal bovine serum, 50 IU/ml penicillin, 50 μg/ml streptomycin and 400 μg/ml G418. Membranes from each cell type were prepared by homogenizing the cells in buffer containing 15 mM Tris–HCl pH 7.5, 2 mM $MgCl_2$, 0.3 mM EDTA and 1 mM EGTA, followed by two consecutive centrifugation steps at $40{,}000 \times g$ for 25 min separated by a wash in the same buffer. Membranes were resuspended in buffer containing 7.5 mM Tris–HCl pH 7.5, 12.5 mM $MgCl_2$, 0.3 mM EDTA, 1 mM EGTA and 250 mM sucrose. Protein was quantitated using the Bio-Rad protein assay (Hercules, CA, USA). For saturation analyses, 5, 25 or 10 μg membrane protein from CHO-K1 cells expressing the recombinant human α_{2A}-, α_{2B}- or α_{2C}-adrenoceptor, respectively, was incubated with the radioligand, [^{3}H]rauwolscine, in a final volume of 200 μl binding buffer (75 mM Tris–HCl pH 7.4, 12.5 mM $MgCl_2$, 2 mM EDTA) per well of 96 well plates for 1 h at room temperature. Total and nonspecific binding were determined in quadruplicate. Nonspecific binding was defined in the presence of 1–10 μM yohimbine. Competition binding studies were performed using 1–2.5 nM [^{3}H]rauwolscine (0.5 to 2 times the K_d value depending on the receptor) and eight concentrations of cold competitor ligand in triplicate or quadruplicate. Assays were terminated by rapid filtration through GF/C unifilter plates, presoaked with 0.3% polyethylenimine, with five washes of 0.5 ml cold 50 mM Tris–HCl pH 7.4 buffer, using a Packard Filtermate Harvester. After drying, bound radioactivity was determined by liquid scintillation counting (Packard TopCount) with Microscint 20, 50 μl/well.

2.5. Data analysis and statistics

Agonist and antagonist activity in the functional human saphenous vein α-adrenoceptor bioassay was expressed as mean ± S.E.M. and estimated as follows. Agonist activity was demonstrated as the increase in gram tension over baseline and normalized as % KCl maximum. Agonist potency was expressed as a pD_2 ($-\log_{10}$ of the EC_{50}). EC_{50} = agonist concentration causing a half-maximal response and was estimated using linear regression analysis of the concentration response curves. Antagonist activity was represented by the agonist dose ratio ($DR = A'/A$, where A' and A are the agonist EC_{50} values in the presence and absence of the antagonist). Antagonist potency was expressed as an apparent pK_b ($-\log_{10}$ of K_b, Tallarida, 1988) or pA_2 ($-\log_{10}$ of the antagonist concentration producing an agonist dose ratio = 2, Tallarida and Murray, 1981). $K_b = [B]/(A'/A - 1)$, where [B] is the molar concentration of the antagonist tested (Tallarida, 1988). The pA_2 value was estimated by means of the Schild plot analysis of Tallarida and Murray (1981). Apparent pK_b or pA_2 was calculated using individual DR values ≥ 2 from concentrations of antagonist at which DR values were ≥ 2 in at least 2/3 of the experiments. Statistical significance was taken as $P < 0.05$, using Kruskal–Wallis (multiple group comparison) and/or Mann–Whitney U (two group comparison) non-parametric tests.

Binding data were analyzed using GraphPad Prism (GraphPad Software, San Diego, CA). Competition displacement curves were analyzed using a one-site model, which provided the best fit. The affinity values of the competing compounds in the displacement studies are expressed as pK_i values ($-\log_{10} K_i$) where the K_i value is calculated from the IC_{50} (concentration of drug that inhibits 50% of binding) value using the Cheng–Prusoff equation (Cheng and Prusoff, 1973) as follows: $IC_{50} = K_i(1 + S/K_d)$ where S is the radioligand concentration and K_d is the ligand dissociation constant. The K_d (nM) and B_{max} (maximal specific binding in pmol/mg) values for [^{3}H]rauwolscine at the recombinant human α_{2A}-, α_{2B}- and α_{2C}-adrenoceptor, determined as described above, are: recombinant human α_{2A}-adrenoceptor $K_d = 2.63 \pm 1.56$, $B_{max} = 5.83 \pm 1.86$ ($n = 4$); recombinant human α_{2B}-adrenoceptor $K_d = 4.48 \pm 2.45$, $B_{max} = 18.33 \pm 9.2$ ($n = 3$); recombinant human α_{2C}-adrenoceptor $K_d = 0.52 \pm 0.04$, $B_{max} = 2.69 \pm 0.88$ ($n = 3$). Data are expressed as mean ± S.D. from the indicated number of experiments in parentheses.

2.6. Drugs, cloned receptors, membrane preparations and isotopes

HV 723 (α-ethyl-3,4,5-trimethoxy-α-(3-((2-(2-methoxyphenoxy)ethyl)amino)propyl)benzeneacetonitrile) was synthesized by the Chemical Research Department, Schering-Plough Research Institute, (Kenilworth, NJ, USA). Phenylephrine hydrochloride ((*R*)-3-Hydroxy-α-[(methylamino)methyl] benzenemethanol hydrochloride), yohimbine hydrochloride (17-Hydroxy-yohimban-16-carboxylic acid methyl ester hydrochloride), prazosin hydrochloride (1-[4-Amino-6,7-dimethoxy-2-quinazolinyl]-4-[2-furanylcarbonyl]piperazine hydrochloride), WB 4101 hydrochloride (2-(2,6-Dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane hydrochloride) and BHT-920 dihydrochloride (5,6,7,8-Tetrahydro-6-(2-propenyl)-4H-thiazolo[4,5-d]azepin-2-amine dihydrochloride) were obtained from RBI (Natick, MA, USA). ARC 239 dihydrochloride (2-[2-(4-(2-Methoxyphenyl)piperazin-1-yl)ehtyl]-4,4-dimethyl-1,3-(2*H*,4*H*)-isoquinolindione dihydrochloride) and UK 14,304 (5-Bromo-6-(2-imidazolin-2-ylamino)quinoxaline) were obtained from Tocris Cookson (Ballwin, MO, USA).

Phenylephrine, yohimbine, prazosin, WB-4101, BHT-920 and ARC 239 were prepared as concentrated stocks in deionized water and HV 723 was prepared as a concentrated stock in DMSO before dilution to final concentration in the human saphenous vein functional bioassay buffer. UK 14,304 was prepared as a 3.0 mM stock in DMSO and then diluted further in deionized water before

addition to the baths in the functional human saphenous vein assay. The final concentration of DMSO in the bath did not exceed 0.15% in the human saphenous vein UK 14,304 experiments.

CHO-K1 cells stably expressing the recombinant human α_{2A}-, α_{2B}- and α_{2C}-adrenoceptor were purchased from Euroscreen (Brussels, Belgium). The Swissprot accession numbers for the recombinant human α_{2A}- and α_{2C}-adrenoceptor cDNA clones used for the cell transfections are P08913 and P18825, respectively. The Genbank accession number for the recombinant human α_{2B}-adrenoceptor cDNA clone used for cell transfection is M34041. [^{3}H]rauwolscine (71 Ci/mmol) and Basic FlashPlates® were purchased from NEN Life Science Products (Boston, MA, USA). GF/C unifilter plates and Microscint 20 were purchased from Packard (Downers Grove, IL, USA). EDTA, EGTA, $MgCl_2$ and Tris–HCl were purchased from Sigma (St. Louis, MO, USA). Ham's F12 medium, penicillin and streptomycin were purchased from Life Technologies (Rockville, MD, USA). Fetal bovine serum was obtained from Summit Biotechnology (Fort Collins, CO) and G418 from Gemini Bioproducts (Calabasas, CA).

3. Results

3.1. Effects of α-adrenoceptor agonists and antagonists in the human saphenous vein bioassay

The α-adrenoceptor agonists BHT-920 ($pD_2 = 6.7 \pm 0.1$), UK 14,304 ($pD_2 = 7.2 \pm 0.1$) and phenylephrine ($pD_2 = 4.9 \pm 0.1$, Fig. 1) produced concentration dependent contractions in fresh and cryopreserved human saphenous vein. Under these conditions, maximal contractions to BHT-920 and UK 14,304 were approximately 50% of the phenylephrine maximum (Fig. 1). Tissue sensitivity to BHT-920 and phenylephrine was not affected by cryopreservation. The respective pD_2 values obtained in investigations comparing fresh and cryopreserved human saphenous vein were 6.7 ± 0.1 ($n = 17$) vs. 6.8 ± 0.3 ($n = 9$) for BHT-920 and 5.0 ± 0.2 ($n = 3$) vs. 4.8 ± 0.1 ($n = 6$) for phenylephrine (data not shown).

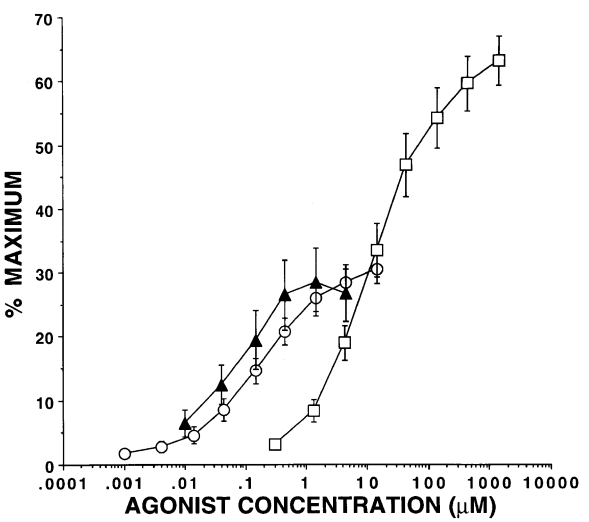

Fig. 1. Contractile responses to BHT-920 (○, $n = 26$), UK 14,304 (▲, $n = 6$) and phenylephrine (□, $n = 9$) in human saphenous vein normalized as the % maximum contraction to 80 mM KCl. Symbols represent the mean ± S.E.M. of responses from the indicated number of experiments using fresh and cryopreserved tissues isolated from 21 (BHT-920), 6 (UK 14,304) and 8 (phenylephrine) patients.

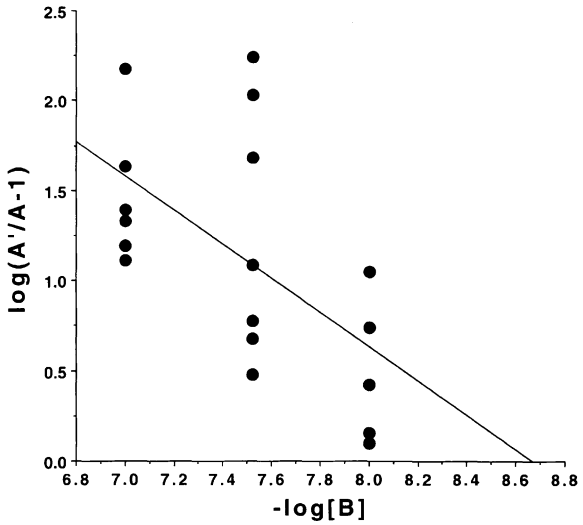

Fig. 2. Schild plot representing the antagonist activity of yohimbine against BHT-920-induced contractions in human saphenous vein. The $pA_2 = 8.7 \pm 0.5$, slope $= -0.93 \pm 0.34$, $r = 0.56$ ($n = 18$). Symbols represent the log of the individual experiment dose ratio − 1 determined using fresh and cryopreserved tissues isolated from eight patients. [B] is the molar concentration of the antagonist.

Human saphenous vein contractions to BHT-920 were competitively blocked by yohimbine ($pA_2 = 8.7$, slope = −0.93, Fig. 2) but not prazosin (0.3 μM, Table 1). In contrast, prazosin blocked phenylephrine-induced contractions ($pK_b = 7.9 \pm 0.2$, Table 1). WB 4101, ARC 239 and HV 723 caused parallel rightward shifts in the concentration response curve to BHT-920. WB 4101 and HV 723 were potent inhibitors (pA_2 or $pK_b \geq 7.7$, Table 1) while ARC 239 was a moderately potent antagonist (Table 1). Similar to yohimbine, Schild plots obtained for WB 4101 and ARC 239 were consistent with competitive antagonist activity (Schild slopes not different from unity, Table 1).

3.2. Competition binding studies

The pK_i values were determined in competition binding studies using membranes from CHO-K1 cells stably expressing the recombinant human α_{2A}-, α_{2B}- and α_{2C}-adrenoceptor. Monophasic displacement curves were observed for each of the competitor compounds tested. Overall, yohimbine was the most potent competitor at the recombinant human α_2-adrenoceptor subtypes with pK_i values ≥ 8.3 (Table 2). In contrast, prazosin exhibited relatively weak displacement at all three subtypes ($pK_i \leq 6.9$, Table 2). ARC 239, WB 4101 and HV 723 demonstrated a range of affinity depending on the receptor subtype. ARC 239 showed a higher affinity for the recom-

Table 1
Antagonist activity (agonist dose ratio) and functional affinity (pA_2 or pK_b) estimates for prazosin, ARC 239, WB 4101 and HV 723 in human saphenous vein

Antagonist	Concentration (μM) (μM)	Agonist	*n*	Antagonist activity		Schild slope
				DR[a]	pA_2 or pK_b	
Prazosin	0.3	PE	3	31.7 ± 10.3[b]	7.9 ± 0.2[c]	–
Prazosin	0.3	BHT-920	3	3.6 ± 2.5	–	–
ARC 239	1.0	BHT-920	10	10.3 ± 3.9[b]	7.0 ± 0.6	-0.94 ± 0.35
	3.0		4	34.8 ± 14.0[b]		
	10.0		6	362.1 ± 255.6[b]		
WB 4101	0.03	BHT-920	6	8.4 ± 5.0[b]	8.3 ± 0.6	-0.92 ± 0.39
	0.1		6	15.3 ± 4.4[b]		
	0.3		5	281.3 ± 252.2[b]		
HV 723	0.3	BHT-920	4	27.4 ± 13.2[b]	7.7 ± 0.3[c]	–

Antagonist activity was evaluated against contractions induced by the type-selective α-adrenoceptor agonists BHT-920 and phenylephrine (PE). Values represent the mean ± S.E.M and *n* = the number of experiments performed using fresh and cryopreserved tissues. Also, *n* = patient number in all cases except the 1.0 μM ARC 239 concentration, where 10 experiments were performed using tissues isolated from nine patients.

[a] Dose ratio.

[b] $P < 0.05$, Kruskal–Wallis and/or Mann–Whitney *U* non-parametric tests.

[c] pK_b estimate.

binant human α_{2B}-adrenoceptor ($pK_i = 8.4$) than the recombinant human α_{2A}- and the recombinant human α_{2C}-adrenoceptor (Table 2). WB 4101 demonstrated equivalent high affinity for the recombinant human α_{2A}- and the recombinant human α_{2C}-adrenoceptor ($pK_i = 8.4$) as compared to its moderate affinity for the recombinant human α_{2B}-adrenoceptor, while HV 723 displayed moderate affinity for each of the receptor subtypes (Table 2). The present binding affinities for yohimbine, WB 4101, ARC 239 and HV 723 at the recombinant human α_{2A}- and α_{2C}-adrenoceptors (Table 2) exhibited a significant linear correlation ($r = 1.0$) when the binding affinities for these agents at α_{2A}- and α_{2C}-adrenoceptors were compared using a correlation analysis (data not shown). The resulting regression slopes, however, differed from unity. The regression slope was 0.7 when the α_{2C}- and α_{2A}-adrenoceptor binding affinities were on the ordinate and the abscissa, respectively (slope = 1.5 when the axes are reversed). In contrast, the yohimbine, WB 4101, ARC 239 and HV 723 binding affinity values at the α_{2A}- or α_{2C}-adrenoceptor showed no intrinsic correlation with the α_{2B}-adrenoceptor subtype binding affinity values ($r \leq 0.33$, data not shown) for these antagonists.

Table 2
Ligand binding to CHO cell membranes expressing single recombinant human α_{2A}-, α_{2B}- or α_{2C}-adrenoceptor subtypes

Ligand	α_{2A}-AR, pK_i	α_{2B}-AR, pK_i	α_{2c}-AR, pK_i
Prazosin	5.8 ± 0.2 (5)	6.7 ± 0.3 (5)	6.9 ± 0.1 (3)
Yohimbine	8.6 ± 0.4 (6)	8.3 ± 0.3 (5)	8.6 ± 0.1 (6)
ARC 239	6.3 ± 0.3 (5)	8.4 ± 0.5 (3)	7.0 ± 0.1 (4)
WB 4101	8.4 ± 0.3 (5)	7.5 ± 0.1 (5)	8.4 ± 0.1 (4)
HV 723	7.5 ± 0.1 (5)	7.4 ± 0.2 (5)	7.9 ± 0.2 (3)

[^{3}H]rauwolscine was used as the radioligand. Values represent the mean ± S.D. with the number of experiments performed shown in parentheses.

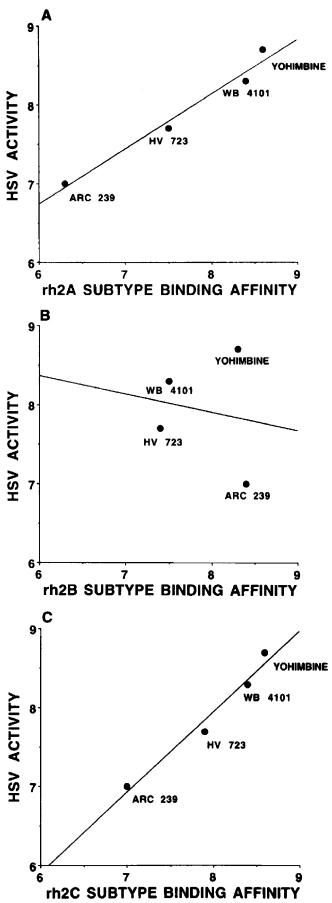

Fig. 3. Correlation analysis comparing the functional activity (pA_2 or pK_b) estimates for yohimbine, WB 4101, ARC 239 and HV 723 in human saphenous vein (HSV) and the competition binding affinity (pK_i) values for the four antagonists in membranes isolated from CHO cells expressing single recombinant human (rh) α_{2A}- (A), α_{2B}- (B) and α_{2C}-adrenoceptor (C) subtypes. Characteristics of the line are slope = 0.7, −0.2 and 1.0 and $r = 0.99$, 0.16 and 0.98, respectively, for the A, B and C regressions.

3.3. Correlation between antagonist activity and binding in human saphenous vein

A strong correlation and a regression slope of unity ($r = 0.98$, slope = 1.0) were obtained when the yohimbine, WB 4101, ARC 239 and HV 723 pA_2 or pK_b estimates from the human saphenous vein bioassay were compared with their respective recombinant human α_{2C}-adrenoceptor binding pK_i values (Fig. 3C). In contrast, the comparisons performed using the pK_i values from the recombinant human α_{2A}-adrenoceptor and the recombinant human α_{2B}-adrenoceptor assays did not support identity. In the case of the recombinant human α_{2A}-adrenoceptor, a high correlation coefficient was returned but the slope differed markedly from unity ($r = 0.99$, slope = 0.7, Fig. 3A). In the case of the recombinant human α_{2B}-adrenoceptor, no correlation was observed (r and slope < 0.2, Fig. 3B).

4. Discussion

Postjunctional α-adrenergic contractile responses of blood vessels are mediated by the α_1- and α_2-adrenoceptor types (Macia et al., 1984; McGrath et al., 1989). In the present investigations, the predominant postjunctional α_2-adrenoceptor subtype of coronary artery bypass graft surgery human saphenous vein was characterized as an α_{2C}-adrenoceptor.

Previous investigations have evaluated the role of the postjunctional α_1- and α_2-adrenoceptor in responses of human saphenous vein derived from coronary artery bypass graft surgery and varicose vein surgery (Docherty and Hyland, 1986; Gavin et al., 1997; Müller-Schweinitzer, 1984; Roberts et al., 1992; Smith et al., 1992; Steen et al., 1984; Weinstein et al., 1989). In the present study using coronary artery bypass graft surgery-derived human saphenous vein, the agonist potency displayed by BHT-920 and UK 14,304 and the antagonist sensitivity of BHT-920 indicated that contractility was mediated by an α_2-adrenoceptor. Additionally, the human saphenous vein contracted to the selective α_1-adrenoceptor agonist phenylephrine, which produced greater peak responses than BHT-920 but was about 100-fold less potent. The present contractile potency estimate for phenylephrine lies within the range of phenylephrine binding affinity found at recombinant α_1-adrenoceptor subtypes (e.g. K_d range = 1.4–47.8 μM, Lomasney et al., 1991). However, this functional potency estimate is somewhat reduced relative to previous functional potency values obtained in human saphenous vein. The previous pEC_{50} values of 5.3 (Steen et al., 1984) and 5.7 (Weinstein et al., 1989) were obtained using methods that differed in the surgical populations used, the portion of the vascular bed studied and tissue storage and assay conditions employed, including the presence of endothelium or neuronal uptake and β-adrenoceptor blockade. Such experimental variables may account for the differences in phenylephrine contractile potency observed in these preparations. Nonetheless, prazosin potently inhibited the phenylephrine contractions in the present investigations, thus indicating activity via the α_1-adrenoceptor. The present finding that the postjunctional α_2- and α_1-adrenoceptors mediate contractility in coronary artery bypass graft surgery human saphenous vein is consistent with earlier studies (Docherty and Hyland, 1986; Roberts et al., 1992; Weinstein et al., 1989). In varicose vein surgery-derived human saphenous vein, the postjunctional α-adrenoceptor contractility was found by others to be mediated predominantly by an α_2-adrenoceptor (Smith et al., 1992; Steen et al., 1984) identified as an α_{2C}-adrenoceptor (Gavin et al., 1997).

Subtype selective agonists or antagonists for the α_{2A}-, α_{2B}- and α_{2C}-adrenoceptor have presently not been identified. Thus, pharmacological identification of α_2-adrenoceptor subtypes has required correlation of cell or tissue α-adrenoceptor agonist and antagonist rank order potency with ligand binding affinity in α_2-adrenoceptor subtype monoreceptor assays (Gavin et al., 1997; Paiva et al., 1999; Rump et al., 1995; Smith et al., 1992; Trendelenburg et al., 1994; Trendelenburg et al., 1997). In the present study, we characterized a postjunctional human saphenous vein α_{2C}-adrenoceptor using the agonist BHT-920 and the α-adrenoceptor antagonist pair ARC 239 and WB 4101. Other studies have demonstrated ARC 239 selectivity for the α_{2C}- and α_{2B}-adrenoceptor subtypes and WB 4101 selectivity for the α_{2C}- and α_{2A}-adrenoceptor subtypes (Parsley et al., 1999; Paiva et al., 1999). Thus, the display of a relatively high potency by both compounds at an α_2-adrenoceptor identifies the α_{2C}-subtype (Parsley et al., 1999). In the present human saphenous vein assay, relatively high potency was displayed by ARC 239 and WB 4101 against BHT-920. These potency estimates for ARC 239 and WB 4101 were consistent with the human α_{2C}-adrenoceptor pK_b estimates of 7.1 and 8.1, respectively, found by Parsley et al. (1999) and the human α_{2C}-adrenoceptor pK_b estimates of 7.2 and 7.9, respectively, found by Gavin et al. (1997). In contrast, other studies have demonstrated relatively low affinity for ARC 239 at the human α_{2A}-adrenoceptor ($pK_i/pK_d \leq 6.3$, Trendelenburg et al., 1997; Smith et al., 1992) and for WB 4101 at the human α_{2B}-adrenoceptor ($pK_i = 7.5$, Smith et al., 1992). In addition to ARC 239 and WB 4101, the α-adrenoceptor antagonist HV 723 was also evaluated against BHT-920 in the present human saphenous vein assay. HV 723 has been shown to be weakly selective for the α_{2C}-adrenoceptor over the α_{2A}- and α_{2B}-adrenoceptor. Others have obtained HV 723 pK_i or pK_b estimates of ≥ 7.5 at the human α_{2C}-adrenoceptor, ≤ 7.2 at the rat α_{2B}-adrenoceptor and 6.6 at the human α_{2A}-adrenoceptor (Gavin et al., 1997; Smith et al., 1992). In the present human saphenous vein bioassay, the potency of HV 723 was consistent with activity at the α_{2C}-adrenoceptor. Taken together, the activities of ARC 239, WB 4101 and HV 723 observed against

BHT-920 in the human saphenous vein bioassay provide strong support for α_{2C}-adrenoceptor mediated contractility.

In this study, the binding affinity (pK_i) estimates obtained for ARC 239, WB 4101 and HV 723 at the recombinant human α_{2A}-, α_{2B}- and α_{2C}-adrenoceptors were consistent with their previously demonstrated subtype selectivities. The present pattern of correlation coefficient and slope that resulted from comparisons of pA_2 or pK_b values for yohimbine, ARC 239, WB 4101 and HV 723 against BHT-920 contractions in human saphenous vein with the recombinant human α_{2A}- and α_{2C}-adrenoceptor subtype pK_i values was the pattern predicted if the contractile receptor was the α_{2C}-adrenoceptor. Furthermore, we observed no correlation between the human saphenous vein pA_2 or pK_b values and the binding affinities at the recombinant human α_{2B}-adrenoceptor subtype. Considered together, the results of the present three correlation analyses indicate that the contractile response to α_2-adrenoceptor agonists is mediated predominantly by a postjunctional α_{2C}-adrenoceptor. The present correlation analysis also concurs with the correlation analysis performed by Gavin et al. (1997) using varicose vein surgery-derived human saphenous vein.

In conclusion, postjunctional α_2-adrenoceptor contractility of human saphenous vein derived from coronary artery bypass graft surgery is mediated predominantly via the α_{2C}-adrenoceptor. This preparation represents a functional bioassay for studying human α_{2C}-adrenoceptor pharmacology.

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