Characterization of α_1 -adrenoceptor subtypes mediating vasoconstriction in human umbilical vein

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1 The present study attempted to characterize pharmacologically the subtypes of α -adrenoceptors mediating contractions in human umbilical vein (HUV).

2 HUV rings were mounted in isolated organ baths and cumulative concentration-response curves were constructed for the α -adrenoceptor agonists phenylephrine and adrenaline. Adrenaline was more potent than phenylephrine ($pD_2=7.29$ and 6.04 respectively).

3 Isoproterenol exhibited no agonism on KCl pre-contracted HUV rings. Propranolol ($1 \mu M$) and rauwolscine ($0.1 \mu M$) did not affect the concentration-response curves to adrenaline. These results demonstrate the lack of involvement of functional β - or α_2 -adrenoceptors in adrenaline-induced vasoconstriction.

4 The non subtype selective α_1 -adrenoceptor antagonist prazosin was evaluated on phenylephrine and adrenaline concentration-response curves. The effects of the competitive α_{1A} and α_{1D} -adrenoceptor antagonists, 5-methyl urapidil and BMY 7378 and the irreversible α_{1B} selective compound chloroethylclonidine (CEC) were also evaluated on adrenaline concentration-response curves.

5 The potencies of prazosin against responses mediated by adrenaline ($pA_2=10.87$) and phenylephrine ($pA_2=10.70$) indicate the involvement of prazosin-sensitive functional α_1 -adrenoceptor subtype in vasoconstriction of the HUV.

6 The potencies of 5-methyl urapidil ($pA_2=6.70$) and BMY 7378 ($pA_2=7.34$) were not consistent with the activation of an α_{1A} - or α_{1D} -adrenoceptor population.

7 Exposure to a relatively low CEC concentration ($3 \mu M$) abolished the maximum response to adrenaline suggesting that this response was mediated by an α_{1B} -adrenoceptor subtype.

8 We conclude that HUV express a prazosin-sensitive functional α_1 -adrenoceptor resembling the α_{1B} -subtype according with the low pA_2 values for both 5-methyl urapidil and BMY 7378 and the high sensitivity to CEC.

Keywords: Human umbilical vein; α_1 -adrenoceptors; adrenaline; isoproterenol; rauwolscine; propranolol; prazosin; BMY 7378; 5-methyl urapidil; chloroethylclonidine

Abbreviations: BK, bradykinin; CEC, chloroethylclonidine; HUV, human umbilical vein

Introduction

The umbilical vein transports the oxygenated blood from the placenta to the foetus, therefore, a normal blood flow is crucial for its growth. Umbilical and placental vessels lack autonomic innervation (Reilly & Russel, 1977; Fox & Khong, 1990). Furthermore, Kawano & Mori (1990) demonstrated that the human umbilical vein (HUV) has no adrenergic innervation and adrenergic nerve fibres are present only in umbilical arteries at the foetal end of the cord. Therefore, HUV is under no influence from the sympathetic nervous system and the regulation of its vascular tone must depend on the release of vasoactive substances which are locally produced or conveyed through the blood stream. Furthermore, it is known that the vasoconstrictor effects of adrenaline and noradrenaline depend on the stimulation of α -adrenoceptors in HUV (Altura *et al.*, 1972).

α_1 -Adrenoceptors are a heterogeneous group of receptors and have been subclassified into α_{1A} -, α_{1B} - and α_{1D} -adrenoceptor subtypes based on radioligand binding, molecular biology and isolated tissue experiments (Hieble *et al.*, 1995). In addition, all three subtypes are expressed in vascular smooth muscle (Price *et al.*, 1994; Vargas & Gorman, 1995; Piascik *et al.*, 1996).

Although the functional affinity values span a broad range, all α_1 -adrenoceptor mediated responses are sensitive to blockade by prazosin, and all show low affinity for the selective α_2 -adrenoceptor antagonist rauwolscine (Bylund *et al.*, 1994; Hieble *et al.*, 1995).

The subdivision of α_1 -adrenoceptors into α_{1A} - and α_{1B} -subtypes has been supported by the identification of several antagonists showing at least 100 fold selectivity for the α_{1A} -adrenoceptor, such as 5-methyl urapidil (Gross *et al.*, 1988). Further evidence to support this subdivision was provided by studies which demonstrated that chloroethylclonidine selectively alkylated the α_{1B} -subtype (Han *et al.*, 1987; Minneman *et al.*, 1988).

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On the other hand, a selective α_{1D} -antagonist, BMY 7378, has been described and was shown to have a high affinity in the rat aorta (Saussy *et al.*, 1994). Furthermore, Kenny *et al.* (1995) have shown that in the rat aorta, BMY 7378 affinity, correlates well with binding affinities at cloned human α_{1D} -adrenoceptor but not with α_{1A} - or α_{1B} -subtypes.

The aim of the present work was to pharmacologically characterize the α -adrenoceptor subtypes mediating contractions in the human umbilical vein in light of current knowledge and the selective ligands available.

Methods

Preparation of the tissues for tension measurements

Approximately 15–35 cm segments were excised from human umbilical cords ($n=140$) midway between the placenta and infant. These cords were obtained from normal full-term deliveries and immediately placed in modified Krebs solution at 4°C (of the following mM composition: NaCl 119, KCl 4.7, NaHCO₃ 25, KH₂PO₄ 1.2, CaCl₂ 2.5, MgSO₄ 1.0, EDTA 0.004, D-glucose 11 and ascorbic acid 0.11).

The veins (internal diameter approximately 5 mm) were dissected out of the cords and cut into rings of approximately 3 mm width. Typically, four rings were taken from each umbilical vein. The preparations were suspended in 10 ml organ baths and stretched with an initial tension of 3–5 g as described previously (Elgoyhen *et al.*, 1993). The time from delivery until the tissue was set up in the organ baths was approximately 3 h. Changes in tension were measured with Grass isometric transducers (FT 03C, Grass Instrument, Quincy, MA, U.S.A.) and displayed on Grass polygraphs (Model 7D). The Krebs solution was maintained at 37°C and at pH 7.4 by constant bubbling with 95% O₂/5% CO₂. During the incubation period, the bath solution was replaced every 15 min with fresh Krebs. After 70 min of equilibration each preparation was contracted with 40 mM KCl and washed. This procedure was designed to test for the functional state of the tissue.

Adrenoceptor agonists studies

Cumulative concentration-response curves to either adrenaline or phenylephrine were obtained after a 120 min equilibration period. In all experiments, bradykinin (BK, 0.3 μ M) was applied at the end of each experiment in order to determine the tissue maximum response (Sardi *et al.*, 1997). In another series of experiments, human umbilical vein rings were contracted with 40 mM KCl after an equilibration period of 120 min. Then, in order to evaluate the possible relaxant effects of a β -adrenoceptor agonist, cumulative-concentration curves to isoproterenol were obtained.

Adrenoceptor antagonists studies

The procedure followed was essentially the same as in the α -adrenoceptor agonist protocol. Each antagonist was applied 30 min before the cumulative addition of agonists (adrenaline or phenylephrine), to ensure that equilibrium was obtained. Experiments were performed in parallel in rings from the same vein. The competitive adrenoceptor antagonists used were propranolol (1 μ M), rauwolscine (0.1 μ M), prazosin (0.03–1 nM), 5-methyl urapidil (1–10 μ M) and BMY 7378 (0.1–1 μ M).

Effects of chloroethylclonidine

The effect of the irreversible antagonist chloroethylclonidine (CEC) was also evaluated. Umbilical rings were exposed to CEC (1–3 μ M) for a period of 30 min and then washed out (bathing fluid was changed twice every 5 min) for 30 min before cumulative concentration-response curves to adrenaline were obtained.

Drugs

Phenylephrine hydrochloride, adrenaline bitartrate, isoproterenol bitartrate, prazosin hydrochloride, BMY 7378 dihydrochloride, chloroethylclonidine dihydrochloride, 5-methyl urapidil and rauwolscine hydrochloride, were purchased from Research Biochemical Incorporated (Natick, MA, U.S.A.). ($\pm$)-Propranolol hydrochloride was purchased from the Sigma Chemical Company (St. Louis, MO, U.S.A.).

All concentrations of drugs are expressed as a final concentration in the organ bath. Preparation of all stock solutions and their subsequent dilution were made in distilled water, except for prazosin and adrenaline. Prazosin was initially dissolved in warmed HCl (0.1 N) then diluted in distilled water. Adrenaline was dissolved in HCl (0.01 N) then diluted in ascorbic acid Krebs solution to avoid oxidation. Stock solutions were stored frozen in aliquots and thawed and diluted daily.

Statistics

All data are presented as means $\pm$ s.e.mean. The number (n) of rings and veins is denoted: number rings / number veins. The responses were calculated as a percentage of the maximum in the cumulative concentration-response curves to adrenaline or phenylephrine. In CEC experiments, responses are expressed as g of developed contraction.

The pD₂ values, negative logarithm of the agonist concentration that produce 50% of the maximum effect, were determined using Graph Pad Prism Version 2.0 (Graph Pad Software Inc., San Diego, CA, U.S.A.). Antagonist pA₂ values and slopes of Schild regressions were calculated as described by Arunlakshana & Schild (1959). Statistical analysis was performed by means of unpaired Student's *t*-test. *P* values lower than 0.05 were taken to indicate significant differences between means.

Results

After a 120 min incubation period, phenylephrine as well as adrenaline produced a concentration-related contraction of human umbilical vein (HUV) rings. However, it should be noted that not every ring would always respond to adrenaline or phenylephrine. In this study 23 of 140 (16%) human umbilical veins did not show adrenergic responses. Adrenaline was more potent than the α_1 -selective agonist phenylephrine. The pD₂ of adrenaline was 7.29 ± 0.06 ($n=13/13$) (n =rings/veins) and 6.04 ± 0.09 ($n=12/12$) for phenylephrine ($P<0.01$). The maximum response was 8.2 ± 1.3 g ($72 \pm 7\%$ of BK_{max}) for phenylephrine and 11.8 ± 1.0 g ($78 \pm 3\%$ of BK_{max}) for adrenaline.

Increasing concentrations of the α_1 -adrenoceptor antagonist prazosin (0.1, 0.3, and 1 nM) produced a parallel rightward shift of the phenylephrine concentration-response curve without affecting the maximum response, indicative of competitive antagonism. Analysis of the data by Schild

regression gave a slope (0.97 ± 0.19) which was not significantly different from unity and a pA_2 value of 10.70 ± 0.17 ($n = 17/12$, Figure 1a). The effect of prazosin on the contractile response to adrenaline was also evaluated. Increasing concentrations of the antagonist (0.03, 0.1, and 0.3 nM) produced a competitive shift of the adrenaline concentration-response curve. Analysis of the data by Schild regression gave a slope (1.28 ± 0.29) not significantly different from unity and a pA_2 value of 10.87 ± 0.13 ($n = 22/13$, Figure 1b). On the other hand, neither the pD_2 nor maximum contraction of the umbilical vein to adrena-

line were affected by the α_2 -adrenoceptor antagonist rauwolscine ($0.1 \mu M$, $n = 6/6$; data not shown) and the β -adrenoceptor antagonist propranolol ($1 \mu M$, $n = 4/4$; data not shown). In addition, the β -adrenoceptor agonist isoproterenol ($0.1 \text{ nM} - 100 \mu M$) did not elicit any relaxant effect on KCl submaximum pre-contraction ($8.4 \pm 1.1 \text{ g}$, $n = 5/5$; data not shown).

The α_{1A} -adrenoceptor antagonist, 5-methyl urapidil ($1, 3$ and $10 \mu M$) produced rightward shifts of the concentration-response curves to adrenaline, with no depression of the maximum response. Analysis of the data by Schild regression

Figure 1 (a) Concentration-response curves and Schild plot for antagonism of phenylephrine induced contraction by prazosin (0.1, 0.3, and 1 nM). (b) Concentration-response curves and Schild plot for antagonism of adrenaline induced contraction by prazosin (0.03, 0.1, and 0.3 nM). Each symbol represents the mean and vertical lines show s.e.mean of at least four separate experiments. Schild plots were constructed with concentration-ratios from individual experiments. The estimates were: (a) $pA_2 = 10.70 \pm 0.17$, slope = 0.97 ± 0.19 , $r = 0.98$ ($n = 17/12$); and (b) $pA_2 = 10.87 \pm 0.13$, slope = 1.28 ± 0.29 , $r = 0.97$ ($n = 22/13$).

gave a slope which was not significantly different from unity (1.08 ± 0.26) and a pA_2 value of 6.70 ± 0.13 ($n = 25/17$, Figure 2).

The α_{1D} -adrenoceptor antagonist, BMY 7378 (0.1, 0.3 and $1 \mu\text{M}$) produced rightward shifts of the concentration-response curves to adrenaline, without reducing the maximum responses over the ranges used for analysis. Analysis of the data by Schild regression gave a slope which was not significantly different from unity (1.14 ± 0.15) and a pA_2 value of 7.34 ± 0.14 ($n = 12/9$, Figure 3).

Exposure to the irreversible antagonist chloroethylclonidine (CEC, $1 \mu\text{M}$) decreased the maximum response to adrenaline in seven of ten rings ($P < 0.05$). In the others, it caused a complete suppression of the response to adrenaline (data not shown). Furthermore, CEC ($3 \mu\text{M}$) completely abolished the response in all experiments ($n = 8/8$, Figure 4). On the other hand, CEC treatment neither exhibited agonism nor modified the maximum response to bradykinin (control, 17.3 ± 2 g; treated, 15.7 ± 2 g).

Figure 2 Concentration-response curves and Schild plot for antagonism of adrenaline induced contraction by 5-methyl urapidil (1, 3, and $10 \mu\text{M}$). Each symbol represents the mean and vertical lines show s.e.mean of at least eight separate experiments. Schild plot was constructed with concentration-ratios from individual experiments. The estimates were: $pA_2 = 6.70 \pm 0.13$, slope = 1.08 ± 0.26 , and $r = 0.97$ ($n = 25/17$).

Figure 3 Concentration-response curves and Schild plot for antagonism of adrenaline induced contraction by BMY 7378 (0.1, 0.3, and $1 \mu\text{M}$). Each symbol represents the mean and vertical lines show s.e.mean of at least three separate experiments. Schild plot was constructed with concentration-ratios from individual experiments. The estimates were: $pA_2 = 7.34 \pm 0.14$, slope = 1.14 ± 0.15 , and $r = 0.99$ ($n = 12/9$).

Table 1 Summary of published affinity data for α_1 -adrenoceptors antagonists. Comparison with data obtained in this study

Competitive antagonists	pK_i on cloned human α -adrenoceptors expressed in cells			pA_2 on contractile response in HUV ^d
	α_{1A}	α_{1B}	α_{1D}	
Prazosin ^a	9.6 ± 0.2	9.5 ± 0.1	9.8 ± 0.3	10.9 ± 0.1
5-Methyl urapidil ^b	8.6 ± 0.1	6.7 ± 0.1	7.6 ± 0.2	6.7 ± 0.1
BMV 7378 ^c	6.4 ± 0.2	7.0 ± 0.3	8.8 ± 0.6	7.3 ± 0.1
Irreversible antagonist	% Inactivation			Sensitivity in HUV
	α_{1A}	α_{1B}	α_{1D}	
CEC ^e 100 μ M, 20 min, 37°C	+	++++	+++	
CEC ^f 3 μ M, 30 min, 37°C				++++

^{a,b} pK_i values represent means $\pm$ s.e.mean of data taken from Forray *et al.* (1994); Goetz *et al.* (1995) and Kenny *et al.* (1995). ^c pK_i values taken from Kenny *et al.*, and Goetz *et al.*, 1995. ^d pA_2 values were obtained using Schild analysis between each antagonist versus adrenaline. ^eSensitivity to CEC estimated as percentage of inactivation in ligand binding experiments: α_{1A} : 18%, α_{1B} : 91%, α_{1D} : 74% Forray *et al.* (1994); α_{1A} : 18%, α_{1B} : 98%, α_{1D} : 75% Schwinn *et al.* (1995). ^fCEC completely abolished the response to adrenaline in HUV.

Figure 4 Effect of chloroethylclonidine (CEC) on adrenaline concentration-response curves. Each symbol represents the mean and vertical lines show s.e.mean of at least seven separate experiments. ^aCEC abolished the response to adrenaline in three of ten experiments. The maximum response to adrenaline in tissues exposed to CEC 1 μ M was significantly different from control (Student's *t*-test, $P < 0.05$).

Discussion

The objective of this study was to perform a pharmacological characterization of the α -adrenoceptor subtypes mediating contractions in the human umbilical vein (HUV) by use of both selective agonists and antagonists. Previous studies have demonstrated the existence of α -adrenoceptors in these vessels (Altura *et al.*, 1972).

Phenylephrine, the non-subtype selective α_1 -agonist produced concentration-dependent contractions 23.8 fold less potent than adrenaline. Prazosin, a non-subtype selective α_1 -adrenoceptor antagonist, competitively inhibited contractile responses induced by phenylephrine and adrenaline in HUV. The pA_2 values for prazosin versus both agonists are consistent with responses being mediated through α_1 -adrenoceptors.

In this tissue, the lack of relaxant effect observed with isoproterenol shows the absence of functional β -adrenoceptors. Furthermore, the potency to adrenaline as well as the maximum response produced did not change with propranolol

(1 μ M) or rauwolsine (0.1 μ M) confirming the absence of both functional β and α_2 -adrenoceptors.

According to these results, adrenaline was considered better than phenylephrine as a pharmacological tool in order to characterize the α_1 -adrenoceptor subtypes mediating contractions in HUV.

5-Methyl urapidil has been widely used for the classification of α_1 -adrenoceptors (Forray *et al.*, 1994; Schwinn *et al.*, 1995; Testa *et al.*, 1995). Its affinity for the α_{1A} -subtype (pK_i approximately 8.6, Table 1) is approximately 10 fold greater than for the α_{1D} -subtype (pK_i approximately 7.6, Table 1) and some 100 fold greater than for the α_{1B} -subtype (pK_i approximately 6.7, Table 1). In this study, the estimated affinity of 5-methyl urapidil in the HUV ($pA_2 = 6.7$) was similar and close to the value expected for an interaction at the cloned α_{1B} -adrenoceptor.

Goetz *et al.* (1995) and Kenny *et al.* (1995) have demonstrated that BMV 7378 is a selective α_{1D} -adrenoceptor antagonist (pK_i approximately 8.8, Table 1) exhibiting about 100 fold selectivity over α_{1A} and α_{1B} -subtypes (pK_i approximately 6.4 and 7.0 respectively, Table 1). In the HUV the estimated affinity ($pA_2 = 7.3$, Table 1) of this antagonist was not consistent with its affinity for cloned α_{1D} -subtype, but is according with the value expected for an interaction with the α_{1B} -subtype.

The α_1 -adrenoceptor subtypes exhibit different sensitivities to the alkylating effects of CEC (Perez *et al.*, 1991; Forray *et al.*, 1994; Schwinn *et al.*, 1995). Both α_{1B} and α_{1D} -adrenoceptor subtypes are effectively inactivated by CEC-treatment, the former subtype being relatively more sensitive (Forray *et al.*, 1994; Schwinn *et al.*, 1995). Although species-dependent variations in sensitivity have been obtained (García-Sainz *et al.*, 1992), α_{1A} -subtype is considerably more resistant to this alkylating agent. In HUV the effects of a 30 min exposure to 1 μ M CEC caused a significant depression of the adrenaline concentration-response curves. Increasing the concentration of CEC (3 μ M) resulted in complete abolition of the contractile response to adrenaline (Table 1). Membrane preparations from cells stably transfected with the cloned human α_{1B} -adrenoceptor gene showed approximately 100% inactivation in radioligand binding studies at a CEC concentration 30 fold greater than the one employed in HUV, where the α_1 -adrenergic response was abolished (Table 1). CEC treatment had no effect on maximum responses to bradykinin at the end of each experiment demonstrating no effect on the tissue

functional state. On the other hand, it was reported that CEC functions as an irreversible α_2 -adrenoceptor agonist (Bultmann & Starke, 1993). CEC treatment did not produce any contractions in HUV in the present study. The lack of contractions suggests the absence of functional α_2 -adrenoceptors in this tissue.

In summary, the absence of both functional β - and α_2 -adrenoceptors has been demonstrated in HUV based on the following observations. Firstly, isoproterenol failed to produce any relaxant effect on KCl pre-contraction. Secondly, propranolol as well as rauwolscine did not shift the concentration-response curve to adrenaline. Thirdly, CEC

alone did not cause direct contractions. On the other hand, the high potency of prazosin, is consistent with a population of α_1 -adrenoceptors mediating contractions in HUV. Furthermore, the very low affinity of 5-methyl urapidil, the low affinity of BMY 7378 and the high sensitivity to CEC, provide pharmacological evidence that α_1 -adrenoceptor agonists-induced contractions in HUV are mediated by α_{1B} -subtypes.

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