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Heterogeneity and complexity of α_1-adrenoceptors in the ovine uterine artery and umbilical vein

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Abstract

To understand the subtypes of α_1-adrenoceptors in the regulation of uterine and umbilical vascular function, the subtypes of α_1-adrenoceptors in the ovine uterine artery and umbilical vein were investigated pharmacologically. The use of the irreversible α_{1B}-adrenoceptor antagonist, chloroethylclonidine, revealed the heterogeneity of α_1-adrenoceptors in these two tissues. Chloroethylclonidine showed different patterns of action. While it depressed the maximal contraction to norepinephrine in the umbilical vein, it did not decrease the maximal response in the uterine artery. The α_{1A}-adrenoceptor antagonist, 2-(2,6-dimethoxyphenoxyethyl) aminomethyl-1,4-benzodioxane (WB 4101), competitively inhibited norepinephrine-induced contractile responses in the ovine uterine artery and umbilical vein with intermediate pA_2 values of 8.30 and 8.45, respectively. Combined use of chloroethylclonidine with either prazosin or WB 4101 produced an additive inhibition of norepinephrine-induced contractions in both tissues, suggesting an interaction of WB 4101 with a chloroethylclonidine-insensitive α_1-adrenoceptor. However, the chloroethylclonidine-insensitive α_1-adrenoceptor differed on the affinity for prazosin in the uterine artery and umbilical vein. The Ca^{2+} channel blocker, nifedipine, inhibited contractions to both the chloroethylclonidine-sensitive α_1-adrenoceptor (α_{1B} subtype) and the chloroethylclonidine-insensitive α_1-adrenoceptor in both tissues. Prazosin, WB 4101 and chloroethylclonidine all inhibited norepinephrine-induced contraction due to the release of calcium from intracellular stores in both tissues. Our results suggest that there is heterogeneity and complexity of α_1-adrenoceptors in the ovine uterine artery and umbilical vein. Both the chloroethylclonidine-sensitive and -insensitive α_1-adrenoceptor may use both intracellular and extracellular Ca^{2+} sources.

Keywords: α_1-Adrenoceptor, subtype; Ca^{2+}; Uterine artery; Umbilical vein

1. Introduction

α_1-Adrenoceptors are members of a large family of G-protein-coupled membrane receptors and play an important role in the regulation of cardiovascular function. α_1-Adrenoceptors are not homogeneous and have been subclassified into α_{1A}-, α_{1B}- and α_{1D}-adrenoceptor subtypes based on functional studies and molecular cloning techniques (Bylund et al., 1994; Hieble et al., 1995). WB 4101 (2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane) and chloroethylclonidine have frequently been used as pharmacological tools to assist in the subclassification of α_1-adrenoceptors (Minneman, 1988). Those adrenoceptors which are chloroethylclonidine-insensitive and have a high affinity for WB 4101 are designated as α_{1A}-adrenoceptors, while those which are chloroethylclonidine-sensitive and have a low affinity for WB 4101 are classified as α_{1B}-adrenoceptors. Activation of α_1-adrenoceptors is associated with Ca^{2+} influx into the cell and Ca^{2+} release from intracellular stores (Ruffolo et al., 1991; Minneman, 1988). It was suggested that α_1-adrenoceptor subtypes use different signaling mechanisms to increase intracellular Ca^{2+} (Han et al., 1987a; Minneman, 1988). α_{1A}-Adrenoceptors stimulate Ca^{2+} influx into the cell through voltage-operated Ca^{2+} channels, while α_{1B}-adrenoceptors mobilize Ca^{2+} from intracellular stores. Molecular cloning technique has suggested the existence of three subtypes of α_1-adrenoceptors: α_{1a}, α_{1b} and α_{1d} (Bylund et al., 1994; Hieble et al., 1995). All three subtypes are expressed in vascular smooth muscle (Piascik et al., 1994; Price et al., 1994; Vargas and Gorman, 1995). Accumulating evidence indicates that the cloned α_{1a}- and α_{1b}-adrenoceptor sub-

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types have the pharmacological properties of the classically defined α_{1A}- and α_{1B}-adrenoceptor subtypes, respectively, while the cloned α_{1d} defines a novel α_{1D}-adrenoceptor subtype (Bylund et al., 1994; Hieble et al., 1995).

Blood flows in the uterine artery and umbilical vein are crucial for the growth of the fetus. The uterine artery transports blood rich in O_2 and nutrients to the placenta where O_2 and nutrients are taken up by the fetal blood and CO_2 is discharged into the maternal circulation. The umbilical vein transports the oxygenated blood from the placenta to the fetus. Although the uterine artery is innervated by adrenergic nerve fibers, degeneration occurs during pregnancy (Sigger et al., 1986). On the other hand, umbilical vessels are not innervated (Fox and Khong, 1990; Sheppard and Bishop, 1973). Therefore, the uterine artery during pregnancy and the umbilical vein are somewhat similar in that they are under little or no influence from the sympathetic nervous system. However, in response to α_1-adrenoceptor agonists, both vessels contract via activation of α_1-adrenoceptors (Isla and Dyer, 1990; Zhang and Dyer, 1991). In vivo and in vitro studies have demonstrated the participation of α_1-adrenoceptor subtypes in the regulation of peripheral vascular function (Piascik et al., 1990; Han et al., 1990). However, the physiological function of possible α_1-adrenoceptor subtypes in the ovine uterine artery and umbilical vein remains to be clarified. In order to understand the role of α_1-adrenoceptor subtypes in the regulation of uterine and umbilical vascular function, uterine arteries and umbilical veins were obtained from near term pregnant sheep and the subtypes of α_1-adrenoceptors were characterized by using pharmacological methods.

2. Materials and methods

2.1. Tissue preparation

Adult pregnant mixed breed sheep near term (130–145 days of gestation) were killed with an injection of pentobarbital sodium. Uterine arteries and umbilical cord were carefully removed without stretching and placed in a modified Krebs' solution of the following composition (mM): NaCl, 115; KCl, 4.70; $CaCl_2$, 1.80; $MgSO_4$, 1.16; KH_2PO_4, 1.18; $NaHCO_3$, 22.14; dextrose, 7.88. EDTA (0.03 mM) was added to suppress oxidation of amines. The uterine artery and umbilical vein were cleaned of connective tissues and cut into 3–4 mm ring segments. The ring segments were mounted between two wires in 10 ml organ baths containing Krebs' solution maintained at 37°C. The Krebs' solution was aerated with a mixture of 95% O_2/5% CO_2. One wire was attached to a fixed support while the second wire was connected to a Grass FT 03 transducer and contractions were recorded by a Grass polygraph (model 7) or a Beckman polygraph (model R611). The segments were equilibrated under 2 g or 1.5 g resting tension for the uterine arteries and umbilical veins, respectively, over 60–90 min with regular replacement of the bath fluid at 20 min intervals. In preliminary experiments, 2 g (uterine artery) and 1.5 g (umbilical vein) resting tension were found to provide optimal tension development to 60 mM KCl. The ring segments were then stimulated with 10 μM norepinephrine. Following complete washout of the agonist and return to baseline, desipramine (100 nM), corticosterone acetate (10 μM) and propranolol (1 μM) were included in the Krebs' solution to block neuronal uptake, extraneuronal uptake and β-adrenoceptors, respectively. These agents were in contact with the tissues for 30 min before a protocol began and throughout the protocol. Concentration–response curves were generated by the cumulative addition of the agonist in approximately one-half log increments, and the response to each concentration of agonist was allowed to stabilize before the next addition.

2.2. Effects of chloroethylclonidine on norepinephrine-induced contractions

For studies in which the irreversible α_{1B}-adrenoceptor-alkylating agent chloroethylclonidine was used, a control concentration–response relationship was first constructed for norepinephrine. After complete washout of the agonist, the tissues were then exposed to chloroethylclonidine (50 or 100 μM) for 30 min, followed by complete washout of the antagonist for 60 min at 15-min intervals. Subsequently, a second concentration–response relationship was obtained for norepinephrine.

2.3. Determination of pA_2 values for WB 4101

A control concentration–response relationship for norepinephrine was constructed in the absence of WB 4101. Following complete washout of the agonist, one of three concentrations (10, 30 and 100 nM) of WB 4101 was added to three organ baths and allowed to equilibrate with the tissues for 30 min before obtaining a second concentration–response relationship to norepinephrine in the presence of the antagonist. pA_2 values ($pA_2 = -\log K_B$) were determined as described by Arunlakshana and Schild (1959). EC_{50} values for the agonist in the absence (EC_{50}) and presence of the antagonist (EC'_{50}) were used to calculate the concentration ratio (CR), ($CR = EC'_{50}/EC_{50}$), which was corrected for time-dependent change as described below, and a plot of log ($CR - 1$) against $-\log$ [antagonist] was made to obtain the pA_2 value ($X-$ intercept) and slope of the regression line.

2.4. Effects of the combined use of chloroethylclonidine with prazosin, WB 4101 or nifedipine

In this protocol, a control concentration–response relationship to norepinephrine was obtained, followed by

washout of the agonist from the bath. Subsequently, tissues were exposed to chloroethylclonidine (50 μM, 30 min), followed by washout of the antagonist for 60 min at 15-min intervals. The tissues were then incubated in the presence or absence of prazosin (10 nM), WB 4101 (10 nM) or nifedipine (1 μM) for 30 min before obtaining a second concentration–response relationship to norepinephrine. The dissociation constants (K_B) for prazosin and WB 4101 were calculated using the equation (Furchgott, 1972): $K_B = [B]/(CR - 1)$, where [B] is the concentration of the antagonist, and CR is the concentration ratio described above. When determining K_B values for prazosin and WB 4101 in tissues pretreated with chloroethylclonidine, the EC_{50} values for the agonist in tissues pretreated with chloroethylclonidine in the absence of the antagonist (EC_{50}) and in tissues pretreated with chloroethylclonidine followed by the antagonist (EC'_{50}) were used to calculate the concentration ratio. Nifedipine studies were conducted in a darkened room.

2.5. Correction for changes in tissue sensitivity

In all the above studies, one tissue was run in parallel with the experimental tissues, but received no antagonists and was used to correct for time-dependent changes in agonist sensitivity during the course of the experiment (Furchgott, 1972). The concentration ratio (CR_T), (EC_{50} at time t/EC_{50} at time 0), was determined. The concentration ratio in the presence of antagonist was then adjusted according to the following formula: adjusted CR = CR/CR_T (Isla and Dyer, 1990).

2.6. Effects of α_1-adrenoceptor antagonists on contractions to norepinephrine in the absence of external Ca^{2+}

Tissues were challenged with norepinephrine (10 μM) repeatedly in normal Krebs' solution (1.8 mM Ca^{2+}) until a reproducible response was obtained. The interval between each addition of norepinephrine was 45 min. After complete washout of the agonist and equilibration in normal Krebs' solution for 60 min, the Krebs' solution was replaced with a Ca^{2+}-free Krebs' solution containing EGTA (1 mM). The tissues were washed with Ca^{2+}-free Krebs' solution 3 times over 10 min. An α_1-adrenoceptor antagonist (prazosin, 10 nM or WB 4101, 10 nM) was then added to the bath for 20 min before the tissues were exposed to norepinephrine (10 μM). In a separate tissue, after complete washout of norepinephrine (10 μM) in normal Krebs' solution, the tissues were incubated with chloroethylclonidine (50 μM) for 30 min. The tissue was then washed over 30 min with normal Krebs' solution to remove the chloroethylclonidine. Subsequently, the tissue was washed 3 times with Ca^{2+}-free Krebs' solution containing EGTA (1 mM) over a 10 min period and the incubation in the Ca^{2+}-free Krebs' solution continued for another 20 min. The tissue was then exposed to norepinephrine (10 μM). The contractile response to norepinephrine for each tissue in the normal Krebs' solution was taken as 100%.

2.7. Drugs

(−)-Norepinephrine bitartrate, desipramine hydrochloride, propranolol hydrochloride and corticosterone acetate were purchased from Sigma (St. Louis, MO, USA). WB 4101 and chloroethylclonidine were obtained form Research Biochemicals International (Natick, MA, USA). Nifedipine and prazosin hydrochloride were gifts from Ciba (Summit, NJ, USA) and Pfizer (Brooklyn, NY, USA), respectively.

2.8. Statistics

Results are expressed as means ± S.E. Differences between means were tested for significance using the Student's *t*-test for paired or unpaired data. A P value of less than 0.05 was taken as significant.

3. Results

3.1. Norepinephrine sensitivity; pD_2 ($-log\ EC_{50}$) and time-dependent change of tissue sensitivity of the uterine artery and umbilical vein

Norepinephrine produced concentration-dependent contractions of both the ovine uterine artery and umbilical vein. The potency of norepinephrine in both tissues was similar, although the absolute force was greater in the uterine artery than in the umbilical vein. The tissue sensitivity to norepinephrine as well as the maximal response produced did not change with time during the course of the experiment in either blood vessel (Table 1). The concentration ratios (CR_T) (EC_{50} at time t/EC_{50} at time t) obtained from time control tissues were 1.02 ± 0.07 and 0.97 ± 0.08 in the uterine artery and umbilical vein, respectively.

Table 1
pD_2 values and maximal contractions (E_{max}) for norepinephrine in the uterine artery and umbilical vein

	Control		Time control	
	pD_2	E_{max} (g)	pD_2	E_{max} (g)
Uterine artery	6.54 ± 0.08	31.7 ± 3.3	6.51 ± 0.11	30.5 ± 4.6
Umbilical vein	6.31 ± 0.07	11.8 ± 1.7 [a]	6.34 ± 0.06	12.1 ± 2.4 [a]

In the studies using antagonists, time controls were also run, in which a second control concentration–response to norepinephrine in the absence of antagonists was generated at the same time as was the concentration–response to norepinephrine obtained in the presence of antagonists. E_{max} is expressed as the maximal tension developed in grams. Data are means ± S.E. from 5–7 animals.
[a] $P < 0.05$, comparing to uterine artery.

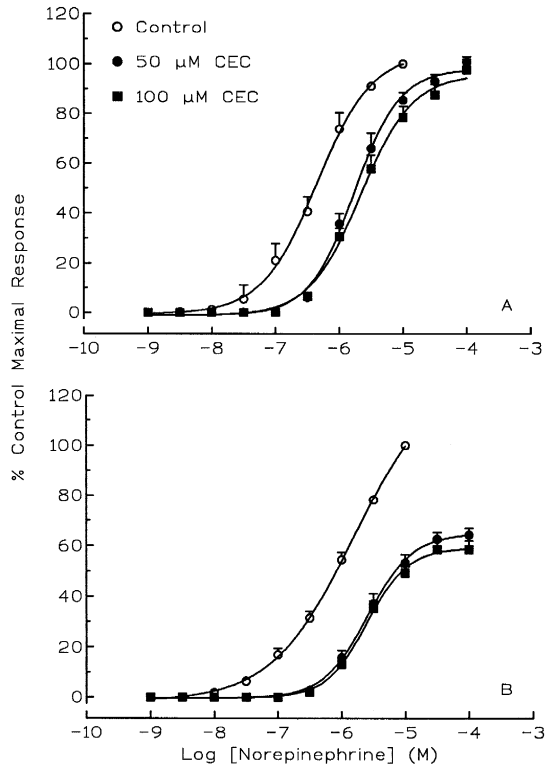

Fig. 1. Concentration–response curves for norepinephrine in the absence and presence of chloroethylclonidine (CEC, 50 and 100 μM) in the ovine uterine artery (A) and umbilical vein (B). The tissues were incubated with chloroethylclonidine for 30 min, followed by washing with fresh Krebs' solution (see Section 2). Each point represents the mean ± S.E. of 5–7 animals.

3.2. Effects of chloroethylclonidine on contractions to norepinephrine

Chloroethylclonidine (50 μM), the selective, irreversible α_{1B}-adrenoceptor antagonist, displaced the concentration–response curve for norepinephrine to the right but did not reduce the maximal response in the uterine artery (Fig. 1A). However, in the umbilical vein, chloroethylclonidine pretreatment significantly depressed the maximal contraction and shifted the concentration–response curve for norepinephrine to the right (Fig. 1B). A higher concentration of chloroethylclonidine (100 μM) did not cause a further rightward shift of the concentration–response curves for norepinephrine, when compared to 50 μM chloroethylclonidine, in either the uterine artery or umbilical vein. Chloroethylclonidine did not produce a contraction in either the uterine artery or the umbilical vein.

3.3. Competitive antagonism by WB 4101

The selective α_{1A}-adrenoceptor antagonist, WB 4101 (10, 30 and 100 nM), shifted the concentration–response curve for norepinephrine to the right in a parallel and concentration-dependent manner in both the uterine artery and umbilical vein. A Schild plot for WB 4101 against norepinephrine yielded a straight line with a slope that was not significantly different from unity for both vessels (−1.07 and −1.09 for the uterine artery and umbilical vein, respectively), suggesting competitive antagonism (Fig. 2). The pA_2 values for WB 4101 against norepinephrine were 8.32 and 8.46 in the uterine artery and umbilical vein, respectively.

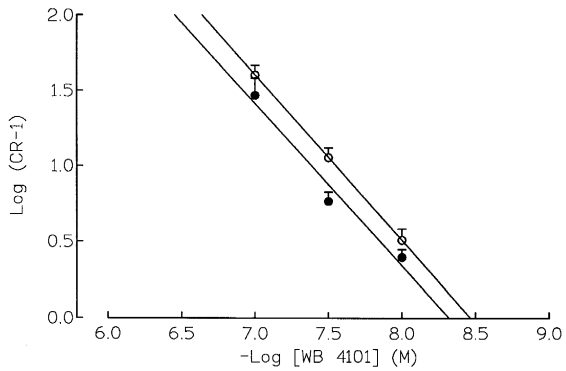

Fig. 2. A Schild plot for the antagonism between WB 4101 and norepinephrine in the ovine uterine artery (●, $pA_2 = 8.32$) and umbilical vein (○, $pA_2 = 8.46$). Each point represents the average of 5–7 animals.

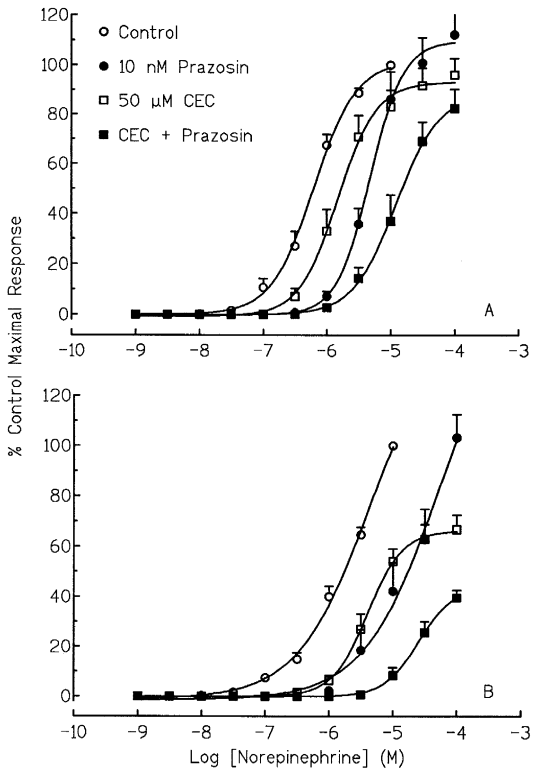

Fig. 3. Effects of the combined use of prazosin (10 nM) with chloroethylclonidine (CEC, 50 μM) on norepinephrine-induced contractions of the ovine uterine artery (A) and umbilical vein (B). Two tissues were pretreated with chloroethylclonidine for 30 min followed by drug washout and one of these tissues was then treated with prazosin for 30 min. A third tissue only received prazosin for 30 min. A concentration–response relationship to norepinephrine was then determined on all tissues. Each point represents the mean ± S.E. of 5 animals.

3.4. Effects of combining chloroethylclonidine with prazosin, WB 4101 or nifedipine

Prazosin (10 nM) alone displaced the concentration–response curve for norepinephrine to the right in both the uterine artery and umbilical vein. The pK_B ($-\log K_B$) values for prazosin against norepinephrine were 8.83 and 9.41 in the uterine artery and umbilical vein, respectively. Pretreatment with chloroethylclonidine (50 μM) followed by prazosin further shifted the concentration–response curve for norepinephrine to the right in both tissues (Fig. 3). The pK_B values for prazosin after chloroethylclonidine pretreatment were 8.81 and 9.39 in the uterine artery and umbilical vein, respectively (see Section 2 for details). Thus, chloroethylclonidine pretreatment did not change the pK_B values for prazosin.

WB 4101 (10 nM) shifted the concentration–response curve for norepinephrine to the right in both the uterine artery and umbilical vein. Pretreatment of these tissues with chloroethylclonidine (50 μM) followed by WB 4101 further shifted the concentration–response curve for norepinephrine to the right in both tissues (Fig. 4). The pK_B values for WB 4101 before chloroethylclonidine pretreatment were 8.24 and 8.52 in the uterine artery and umbilical vein, respectively, which are almost identical to those obtained using the Schild plot method. The pK_B values for WB 4101 after chloroethylclonidine pretreatment (8.22 and 8.58 in the ovine uterine artery and umbilical vein, respectively) remained unchanged, compared to those obtained without chloroethylclonidine pretreatment. The inhibition was additive for the combined use of chloroethylclonidine and WB 4101.

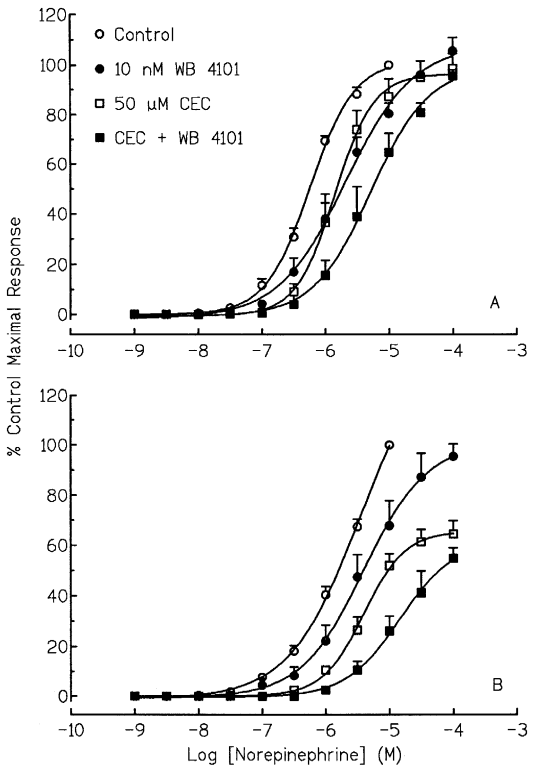

Fig. 4. Effects of the combined use of WB 4101 (10 nM) with chloroethylclonidine (CEC, 50 μM) on norepinephrine-induced contractions of the ovine uterine artery (A) and umbilical vein (B). Two tissues were pretreated with chloroethylclonidine for 30 min followed by drug washout and then one of these tissues was treated with WB 4101 for 30 min. A third tissue only received WB 4101 for 30 min. A concentration–response relationship to norepinephrine was then determined on all tissues. Each point represents the mean ± S.E. of 6 animals.

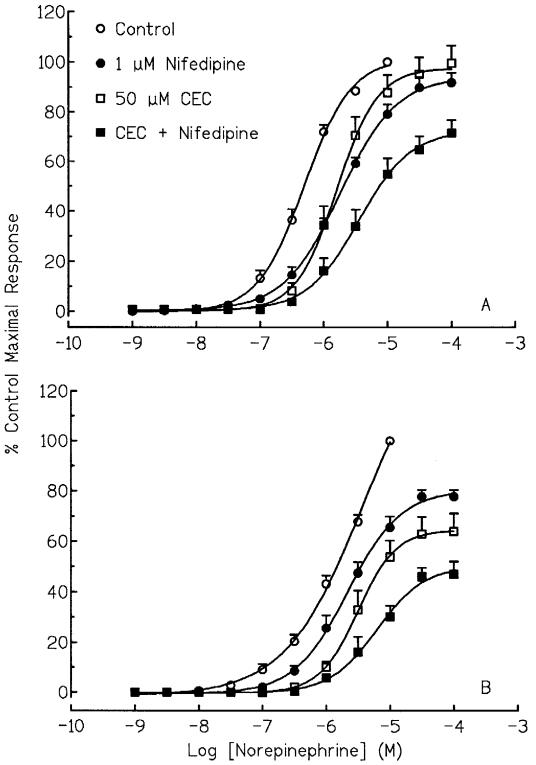

Fig. 5. Effects of the combined use of nifedipine (1 μM) with chloroethylclonidine (CEC, 50 μM) on norepinephrine-induced contractions of the ovine uterine artery (A) and umbilical vein (B). Two tissues were pretreated with chloroethylclonidine for 30 min followed by drug washout and then one of these tissues was treated with nifedipine for 30 min. A third tissue only received nifedipine for 30 min. A concentration–response relationship to norepinephrine was then determined on all tissues. Each point represents the mean ± S.E. of 6 animals.

Inhibition of voltage-operated Ca^{2+} channels by nifedipine (1 μM) shifted the concentration–response curve for norepinephrine to the right without significantly decreasing the maximal response in the uterine artery (Fig. 5A). In the umbilical vein, nifedipine displaced the concentration–response curve for norepinephrine to the right and significantly depressed the maximal contraction to norepinephrine (Fig. 5B). The pD_2 values for norepinephrine in the presence of nifedipine were 5.70 ± 0.08 and 5.53 ± 0.06 for the uterine artery and umbilical vein, respectively. After pretreatment with chloroethylclonidine, nifedipine further shifted the concentration–response curves for norepinephrine to the right in both tissues (Fig. 5). The pD_2 values for norepinephrine in the presence of

nifedipine after chloroethylclonidine pretreatment were 4.94 ± 0.06 and 4.21 ± 0.03 for the uterine artery and umbilical vein, respectively, and these values are significantly different from those obtained from tissues in the presence of nifedipine without chloroethylclonidine pretreatment ($P < 0.05$). The inhibition of responses to norepinephrine by chloroethylclonidine and nifedipine was additive in both the uterine artery and umbilical vein.

3.5. Effects of α_1-adrenoceptor antagonists on contractions to norepinephrine in the absence of external Ca^{2+}

In normal Krebs' solution containing 1.8 mM Ca^{2+}, norepinephrine (10 μM) produced a contraction in both the uterine artery and umbilical vein which was composed of two components: a phasic contraction followed by a tonic contraction. In Ca^{2+}-free Krebs' solution, norepinephrine (10 μM) produced only a transient contraction in both tissues. The contractions in response to norepinephrine (10 μM) in Ca^{2+}-free medium were $42.4 \pm 3.7\%$ and $18.1 \pm 3.2\%$ in the uterine artery and umbilical vein, respectively, of those obtained in normal Krebs' solution (Fig. 6). Prazosin (10 nM), a selective α_1-adrenoceptor antagonist, WB 4101 (10 nM), a selective α_{1A}-adrenoceptor antagonist, and chloroethylclonidine (50 μM), an irreversible α_{1B}-adrenoceptor antagonist, all significantly inhibited contractions to norepinephrine in both the uterine artery and umbilical vein in the absence of external Ca^{2+}. The magnitude of the inhibition was similar for all three antagonists in the uterine artery. However, in the umbilical vein, prazosin and chloroethylclonidine were more potent in inhibiting contractions to norepinephrine.

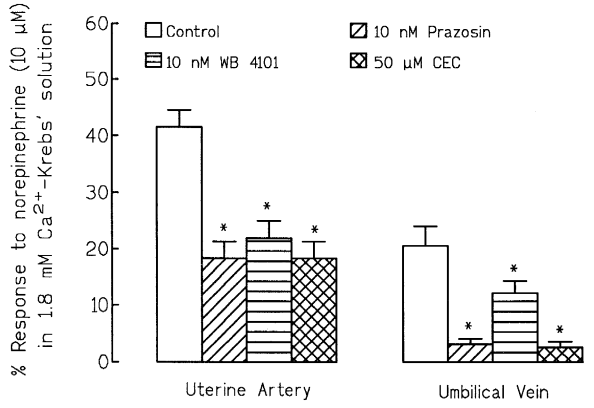

Fig. 6. Effects of α_1-adrenoceptor antagonists (prazosin, WB 4101 and chloroethylclonidine (CEC)) on contractions induced by norepinephrine (10 μM) in the absence of external calcium in the ovine uterine artery and umbilical vein. A contractile response to norepinephrine was obtained in normal Krebs' solution containing 1.8 mM Ca^{2+} followed by drug washout. The tissues were then washed and incubated in Ca^{2+}-free Krebs' solution containing 1 mM EGTA. A contractile response to norepinephrine was then obtained in a control tissue and tissues pretreated with α_1-adrenoceptor antagonists and expressed as percentage of the contractile response in 1.8 mM Ca^{2+}-Krebs' solution. See Section 2 for details. Values are the means ± S.E. of 5–9 animals. * Indicates values that are significantly different form control values obtained in the absence of external calcium, $P < 0.05$.

4. Discussion

Vasoconstriction elicited by α-adrenoceptor agonists in the ovine uterine artery and umbilical vein was previously shown to be mediated by α_1-adrenoceptors (Isla and Dyer, 1990; Zhang and Dyer, 1991). Prazosin, a selective α_1-adrenoceptor antagonist, competitively inhibited contractile responses induced by norepinephrine in both the uterine artery and umbilical vein. The K_B values for prazosin in the present study were similar to those previously reported by our laboratory (Isla and Dyer, 1990; Zhang and Dyer, 1991), confirming the presence of α_1-adrenoceptors in both vessels.

Chloroethylclonidine is an irreversible alkylating derivative of clonidine and possesses different effectiveness in blocking α_1-adrenoceptor subtypes (Hieble et al., 1995; Minneman, 1988). As a pharmacological tool, chloroethylclonidine has been extensively used to differentiate α_1-adrenoceptor subtypes. It has been proposed that those α_1-adrenoceptors which are sensitive to chloroethylclonidine alkylation are of the α_{1B}-adrenoceptor subtype, while those α_1-adrenoceptors which are insensitive to chloroethylclonidine alkylation are of the α_{1A}-adrenoceptor subtype (Minneman, 1988). In addition, recent findings suggested partial sensitivity of the α_{1D}-adrenoceptor to chloroethylclonidine alkylation (Hieble et al., 1995). In the present study, chloroethylclonidine shifted the concentration–response curves for norepinephrine to the right in both the uterine artery and umbilical vein. In the uterine artery, however, chloroethylclonidine did not depress the maximal contraction to norepinephrine. Similar observations have been made in other vascular smooth muscle (Aboud et al., 1993; Oriowo et al., 1992; Oriowo and Ruffolo, 1992; Tian et al., 1990). It remains unclear why chloroethylclonidine depresses the maximal response in some vessels but not in others. Receptor reserve may contribute to the variation of chloroethylclonidine action. However, it is unlikely that receptor reserve has a role in this phenomenon in the uterine artery, since previous studies showed that there was no substantial α_1-adrenoceptor reserve in this tissue (Isla and Dyer, 1990). Recently, the expression and function of the α_{1D}-adrenoceptor was demonstrated in vascular smooth muscle (Piascik et al., 1994, 1995) and that this subtype is inactivated by chloroethylclonidine (Perez et al., 1991; Hieble et al., 1995). It is possible that a mixed population of α_{1B}- and α_{1D}-adrenoceptors may be present in the uterine artery. The inhibitory effects of chloroethylclonidine in the uterine artery and umbilical vein suggest that there is heterogeneity of α_1-adrenoceptors in these two tissues.

Radioligand binding and functional studies using a

competitive α_{1A}-adrenoceptor antagonist, WB 4101, demonstrated two separate populations of α_1-adrenoceptors in rat brain tissues (Han et al., 1987a; Morrow and Creese, 1986). The α_{1A}-adrenoceptor subtype is designated as having high affinity for WB 4101, while the α_{1B}-adrenoceptor subtype has low affinity for WB 4101. In the present study, WB 4101 shifted the concentration–response curves for norepinephrine to the right in a parallel manner. The Schild plot yielded a straight line whose slope was not significantly different from unity in both the uterine artery and umbilical vein, suggesting competitive antagonism at a single site (Furchgott, 1972; Kenakin, 1982). The pA_2 values for WB 4101 in the uterine artery and umbilical vein were 8.32 and 8.46, respectively. These values did not match the pK_i values obtained from either functionally characterized α_{1A}- (9.4) and α_{1B}- (8.0) adrenoceptor subtypes (Morrow and Creese, 1986) or cloned α_{1a}- (9.0), α_{1b}- (8.0) and α_{1d}- (8.9) adrenoceptor subtypes (Schwinn et al., 1995). Similar to our observations, intermediate pA_2 or pK_B values have been reported previously in other vascular beds (Oriowo and Ruffolo, 1992; Oshita et al., 1993; Sayet et al., 1993; Tian et al., 1990).

In order to delineate the α_1-adrenoceptor subtypes which interacted with WB 4101 in the uterine artery and umbilical vein, the combined effects of chloroethylclonidine or prazosin with WB 4101 were explored in these two tissues. The sensitivity to inactivation by chloroethylclonidine differs with α_1-adrenoceptor subtypes (Han et al., 1987b; Hieble et al., 1995; Minneman, 1988). Insensitivity to inactivation by chloroethylclonidine is a well-recognized characteristic of functionally defined α_{1A}-adrenoceptors, and WB 4101 interacted differently with chloroethylclonidine-sensitive and chloroethylclonidine-insensitive α_1-adrenoceptors (Han et al., 1987b). This criterion still holds true for differentiating α_{1B}- and α_{1D}-adrenoceptors from α_{1A}-adrenoceptor (Hieble et al., 1995). The findings of contractile responses to norepinephrine after chloroethylclonidine pretreatment in both the uterine artery and umbilical vein further supports the heterogeneity of α_1-adrenoceptor in these two tissues. First, we addressed whether chloroethylclonidine-resistant contractions in response to norepinephrine in the uterine artery and umbilical vein were sensitive to antagonism by prazosin. After pretreatment with chloroethylclonidine, contractions to norepinephrine were further inhibited by prazosin in both tissues. These observations indicate that the response which was resistant to chloroethylclonidine inactivation was still sensitive to antagonism by prazosin and mediated by α_1-adrenoceptors. Secondly, we investigated whether WB 4101 antagonized chloroethylclonidine-insensitive contraction in the uterine artery and umbilical vein. Our data showed additional antagonism by WB 4101 of tissues pretreated with chloroethylclonidine. Similar pK_B values for WB 4101 obtained without and after chloroethylclonidine pretreatment suggest WB 4101 acts on chloroethylclonidine-insensitive α_1-adrenoceptors. This observation excluded the possible interaction of WB 4101 with α_{1B}-adrenoceptors. Although the pK_B or pA_2 values for WB 4101 were close to the pK_i (approx. 8.6) obtained on the cloned α_{1d}-adrenoceptor (Vargas and Gorman, 1995), the interaction of WB 4101 with chloroethylclonidine-insensitive α_1-adrenoceptor disagreed with the feature of sensitivity to chloroethylclonidine inactivation of the α_{1d}-adrenoceptor. The intermediate affinity for WB 4101 in the uterine artery could suggest the existence of α_{1L}-adrenoceptors as proposed by Muramatsu et al. (1990). However, the α_{1L}-adrenoceptor subtype has a low affinity for prazosin ($pK_d < 9.0$). The pK_B for prazosin was 9.4 in the umbilical vein, which does not fit that proposed for the α_{1L}-adrenoceptor subtype. The results obtained in the present study revealed the heterogeneity of chloroethylclonidine-insensitive α_1-adrenoceptors, as indicated by the affinity for prazosin. However, the results in the present study also suggest that chloroethylclonidine-insensitive α_1-adrenoceptors in the uterine artery and umbilical vein cannot be fully classified by the criteria for α_{1A}, α_{1B}, and α_{1D} or α_{1L} and α_{1N} subtypes. Further studies with the application of a range of antagonists, radioligand binding and molecular biology techniques could help clarify this issue. It is also possible that the chloroethylclonidine-insensitive α_1-adrenoceptor with intermediate affinity for WB 4101 could be a new α_1-adrenoceptor subtype which has not been characterized.

Our results differ from the observations in rat and dog aorta in which neither prazosin nor WB 4101 antagonized the residual contractile responses to norepinephrine after pretreatment with chloroethylclonidine (Oriowo and Bevan, 1990; Oriowo and Ruffolo, 1992). Oriowo and Bevan (1990) suggested that the chloroethylclonidine-resistant contraction to norepinephrine was mediated by a non-adrenoceptor. This is not the case in the uterine artery and umbilical vein, since combined use of chloroethylclonidine with prazosin or WB 4101 produced an additive inhibition of contractile responses to norepinephrine. In addition, Oriowo and Ruffolo (1992) observed that chloroethylclonidine-resistant contractions to norepinephrine were sensitive to antagonism by prazosin and WB 4101 in the rabbit aorta. These observations suggest that the ability of prazosin or WB 4101 to antagonize chloroethylclonidine-resistant contractions to norepinephrine is species/tissue-dependent. The intermediate affinity for WB 4101 in these two tissues could be due to species difference and hormone status. Since the affinity for an antagonist is modulated by the ternary complexes between antagonist, receptor and G protein (Kenakin et al., 1995), the coupling between WB 4101, α_{1A}-adrenoceptor and G protein in the uterine artery and umbilical vein could be altered during pregnancy and could be species/tissue-dependent.

Ca^{2+} source was once used to define α_1-adrenoceptor subtypes (Han et al., 1987a). α_{1A}-Adrenoceptor-mediated responses were considered to be associated with Ca^{2+}

influx into the cell and were sensitive to antagonism by WB 4101 and nifedipine, while α_{1B}-adrenoceptor-mediated responses were thought to be linked to phospholipase C and mobilization of intracellular Ca^{2+} and were sensitive to alkylation by chloroethylclonidine. Contractions induced by agonists in Ca^{2+}-free medium are thought to be the result of release of intracellular stored Ca^{2+} by inositol 1,4,5-trisphosphate ($Ins(1,4,5)P_3$), a product of agonist-stimulated phospholipase C activity (Minneman, 1988). Inhibition of contractions to norepinephrine by chloroethylclonidine in Ca^{2+}-free medium in both the uterine artery and umbilical vein is consistent with the suggestion that the α_{1B}-adrenoceptor is coupled to the release of intracellular Ca^{2+} (Han et al., 1987a; Minneman, 1988). However, inhibition of contractions to norepinephrine by a low concentration of WB 4101 in Ca^{2+}-free medium in both tissues may suggest that the chloroethylclonidine-insensitive α_1-adrenoceptor is also capable of releasing intracellular Ca^{2+}. Similarly, Lepretre et al. (1994) demonstrated that activation of α_{1A}-adrenoceptors, a chloroethylclonidine-insensitive α_1-adrenoceptor, stimulated phosphoinositide hydrolysis and mobilized intracellular Ca^{2+} in the rat portal vein. Our present study also showed that nifedipine and chloroethylclonidine acted in a similar pattern in both the uterine artery and umbilical vein, suggesting the possible coupling of the α_{1B}-adrenoceptor to Ca^{2+} influx. In addition, we also found that the contractile response mediated by chloroethylclonidine-insensitive α_1-adrenoceptor was also sensitive to nifedipine blockade in both tissues. α_1-Adrenoceptor subtypes may not be coupled to only a single transduction pathway (Minneman and Esbenshade, 1994). Wu et al. (1992) observed that all three α_1-adrenoceptor subtypes were coupled to phospholipase C through a pertussis toxin-insensitive Gq protein. In addition, the α_{1B}-adrenoceptor subtype was reported to stimulate Ca^{2+} influx (Han et al., 1992). Our findings suggest the inadequacy of using Ca^{2+} source in the classification of α_1-adrenoceptor subtypes and support previous work which indicated that this is a poor method (Ford et al., 1994).

Chloroethylclonidine was reported to cause contractions in some but not all vascular beds (Oriowo and Bevan, 1990; Tian et al., 1990). However, it did not produce any contractions in the uterine artery and umbilical vein in the present study. It was reported that chloroethylclonidine functions as an irreversible α_2-adrenoceptor agonist (Bultmann and Starke, 1993; Nunes and Guimaraes, 1993). Contractile responses of chloroethylclonidine in some vascular smooth muscle could be the result of the interaction of chloroethylclonidine with α_2-adrenoceptors, while the lack of contractions could be due to the absence of functional α_2-adrenoceptors in the other vascular smooth muscle. In fact, no functional α_2-adrenoceptors were found in the ovine uterine artery and umbilical vein (Isla and Dyer, 1990; Zhang and Dyer, 1991).

Yamaguchi and Kopin (1980) proposed that α_1-adrenoceptors are located at neuroeffector junctions (junctional receptors), while α_2-adrenoceptors are located at extrajunctional sites (extrajunctional receptors). Piascik et al. (1991) further suggested that α_{1A}-adrenoceptors are located junctionally while α_{1B}-adrenoceptors are located extrajunctionally. Since umbilical vessels are not innervated (Fox and Khong, 1990), the finding by Zhang and Dyer (1991) and our observations suggest the possibility of an extrajunctional location for α_{1B}- and possibly α_{1A}-adrenoceptors in the umbilical vein. It is generally accepted that the physiological role of postjunctional α_1-adrenoceptors appears to be responsible for maintaining resting vascular tone, while extrajunctional α_2-adrenoceptors may respond to circulating epinephrine (Ruffolo et al., 1991). Recently, Piascik et al. (1990) proposed that α_{1A}-adrenoceptors appear to play a role in the maintenance of vascular tone, while α_{1B}-adrenoceptors respond to circulating catecholamines. Our findings suggest that both chloroethylclonidine-sensitive (α_{1B} subtype) and chloroethylclonidine-insensitive α_1-adrenoceptors in the umbilical vein could respond to circulating catecholamines and participate in the regulation of resting tone, since there are no apparent α_2-adrenoceptors in the ovine umbilical vein (Zhang and Dyer, 1991).

In summary, the heterogeneity of α_1-adrenoceptors in both the ovine uterine artery and umbilical vein has been demonstrated, based on the sensitivity to inactivation of chloroethylclonidine. In addition, we provide evidence that the chloroethylclonidine-insensitive α_1-adrenoceptor is also heterologous since it possesses different affinity for prazosin. Our data also imply that both the chloroethylclonidine-sensitive α_1-adrenoceptor (α_{1B} subtype) and the chloroethylclonidine-insensitive α_1-adrenoceptor use both extracellular and intracellular Ca^{2+} sources.

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