ELSEVIER

European Journal of Pharmacology 312 (1996) 45–51

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Pharmacological characterization of α_1-adrenoceptors in porcine uterine artery

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Received 24 April 1996; accepted 29 May 1996

Abstract

This study was undertaken to ascertain if changes in the affinity to α_1-adrenoceptor agonists and antagonists could explain the increase in uterine artery vasoconstriction to adrenergic stimuli during the luteal phase of the estrous cycle in pigs. We also sought to determine the subtype(s) of adrenoceptor (α_{1A} and α_{1B}) in the porcine uterine artery. When phenylephrine was the agonist, uterine artery pA_2 values for prazosin were 8.98, 9.04 and 9.10 in the luteal and follicular phases and in early pregnancy, respectively. The K_A (dissociation constant) values for phenylephrine were 6.5, 3.7 and 4.4 (μM) in the luteal and follicular phases and in early pregnancy, respectively. The use of the putative α_{1A}-adrenoceptor WB4101 (2-[[[2-(2,6-dimethoxyphenoxy)ethyl]amino]methyl]-1,4-benzodioxane) and the α_{1B}-adrenoceptor antagonist chloroethylclonidine indicated that both α_{1A}- and α_{1B}-adrenoceptors are present in the porcine uterine artery and that a similar magnitude of inhibition of responses to noradrenaline by each of the antagonists occurred in arteries from the luteal and follicular phases and in early pregnancy. This suggests that the α_{1A} : α_{1B} adrenoceptor ratio does not vary significantly during the estrous cycle and in early pregnancy. This study indicates that porcine uterine artery α_1-adrenoceptor affinity is similar in the luteal and follicular phases of the estrous cycle and in early pregnancy.

Keywords: α_1-Adrenoceptor; Uterine artery; Estrous cycle; Pregnancy

1. Introduction

α-Adrenoceptor agonists, such as phenylephrine and noradrenaline, are effective constrictors of human, ovine and porcine uterine vasculature (Dyer and Gough, 1971; Isla and Dyer, 1990a; Ford et al., 1984). There is evidence that α_1-adrenoceptors can be subdivided into two pharmacologically distinct subtypes, α_{1A} and α_{1B} (Morrow and Creese, 1986; Minneman, 1988). The α_{1A}-adrenoceptor has a higher affinity for the competitive antagonist WB4101 (2-[[[2-(2,6-dimethoxyphenoxy)ethyl]amino]methyl]-1,4-benzodioxane) and is not inactivated by chloroethylclonidine. The α_{1B}-adrenoceptor has a lower affinity for WB4101 and is potently inactivated by chloroethylclonidine. Since chloroethylclonidine distinguishes between the two α_1-adrenoceptors in rat liver, spleen and vas deferens, it has been proposed as a pharmacologic tool to help classify α_1-adrenoceptor subtypes in other tissues (Han et al., 1987a). Another distinguishing characteristic between α_{1A}- and α_{1B}-adrenoceptors is their difference regarding the use of extracellular/intracellular sources of Ca^{2+} in mediating the biologic response. α_{1A}-Adrenoceptors appear to control the influx of extracellular Ca^{2+} into the cell via voltage-dependent Ca^{2+} channels. α_{1B}-Adrenoceptors elevate intracellular Ca^{+2} via the hydrolysis of phosphatidylinositol bisphosphate to inositol (1,4,5) triphosphate and diacylglycerol (Tsujimoto et al., 1989).

While α_1-adrenoceptor agonists are potent vasoconstrictors of the uterine vasculature in pigs (Ford et al., 1984; Guenther et al., 1988), the type of receptor(s) mediating this response is unknown. The purpose of this study was to characterize the α_1-adrenoceptor and its subtypes during the luteal and follicular phases of the estrous cycle and in early pregnancy.

2. Materials and methods

2.1. General considerations

A local abattoir was the source for specimens of porcine uteri. Arteries were obtained from animals in the luteal and

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PII S0014-2999(96)00418-9

follicular phases of the estrous cycle, and in early pregnancy (approximately 40–45 days). The crown-rump measurement (Evans and Sack, 1973) was used to determine the day of pregnancy. The luteal phase was determined by ascertaining the presence of corpora lutea and the follicular phase was determined based on the presence or absence of follicles. Immediately after collection, the tissues were placed in an ice-cold modified Krebs' solution of the following composition (mM): NaCl, 115.21; KCl, 4.70; $CaCl_2$, 1.80; $MgSO_4$, 1.16; KH_2PO_4, 1.18; $NaHCO_3$, 22.14; dextrose, 7.88; EDTA (disodium ethylenediamine tetraacetic acid, 0.03, to suppress the oxidation of amines); indomethacin, 0.001 (Fiscus and Dyer, 1982; to inhibit prostaglandin synthesis) and aerated with air. The tissues were then transported to the laboratory.

The (middle) uterine artery was used to prepare rings of approximately 5 mm in length. The arterial rings were suspended in 10 ml organ baths maintained at 37°C and bathed in the modified Krebs' solution which was continuously aerated with an oxygen : carbon dioxide (95 : 5) mixture. Two stainless steel hooks were passed through the vessel lumen (Hooker et al., 1977). One hook was attached to the base of the bath and the other was attached to a Grass force displacement transducer (FT03) which was in turn connected to a Beckman R-611 8 channel chart recorder. Tissue contractions were recorded isometrically. Tissues not used immediately were stored at 4°C in oxygenated Krebs' solution and used within 24 h. Arterial rings were initially equilibrated in the bath for 30 min under no tension. The tissues were then placed under 4 *g* tension for 30 min (Sato and Aoki, 1991) and then brought to a resting tension of 2 *g* and maintained at this tension for 60 min and during this time the bath fluid was replaced at 15-min intervals.

In preliminary experiments, a complete concentration-response relationship to phenylephrine ($n = 3$) and noradrenaline ($n = 3$) was obtained in tissues from animals in the follicular phase, luteal phase and in early pregnancy (3 animals each), with the highest concentration of each agonist used being 100 μM. However, this led to a significant shift to the right of the second concentration-response curve for these agonists. To avoid the complications of desensitization, the initial exposure of the tissues to agonists was limited to 3 μM for phenylephrine and 10 μM for noradrenaline. The concentration-response curve was linear at the EC_{50} of the initial curve for each of the agonists (Figs. 1 and 3). The plotted control curves in each figure are initial responses for the 'time control' tissue.

2.2. Determination of the dissociation constant (K_B)

Uterine arterial rings were equilibrated for 60 min with iproniazid (0.36 mM), to inhibit monoamine oxidase, for 60 min and then washed for 40 min at 10-min intervals (Isla and Dyer, 1990a). Cocaine (10 μM), corticosterone acetate (1.0 μM), tropolone (10 μM) and propranolol (10 μM) were added for 20 min (to inhibit uptake mechanisms, block catechol-*o*-methyl transferase and inhibit β-adrenoceptors) prior to obtaining a concentration-response relationship to phenylephrine (Furchgott, 1972; Isla and Dyer, 1990a. Phenylephrine was added to the bath in a cumulative manner in approximately one-half log increments (Van Rossum, 1963). After completion of the first concentration-response relationship, the tissue baths were washed for 60 min at 15-min intervals. Three different concentrations of prazosin (3, 10 and 30 nM) were added to the tissue baths for 60 min. A second concentration-response relationship to phenylephrine was obtained in the presence of prazosin. A ring segment prepared from the same uterine artery which received no antagonist was used

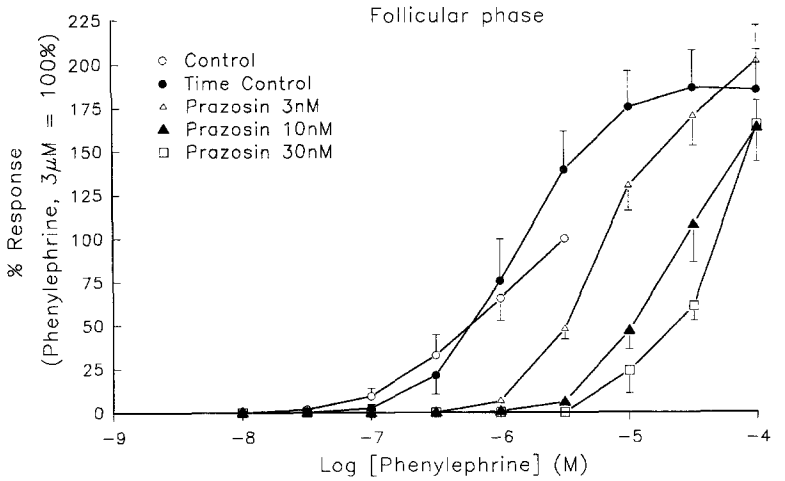

Fig. 1. Cumulative concentration-response curves for phenylephrine of isolated rings of porcine uterine artery (obtained in the follicular phase) before and after equilibration for 60 min in the presence of prazosin (3, 10 and 30 nM). Each point represents the mean ± S.E. of tissues from 6 animals and is expressed as a percentage of the control contraction obtained to phenylephrine (3 μM).

as a 'time control', and was used to correct for any changes in tissue sensitivity during the course of the experiment.

The magnitude of the shift of the log concentration-response relationship to phenylephrine in the presence of prazosin was determined at the EC_{50} of the control response. This shift is referred to as the concentration ratio (CR). Any shift in the phenylephrine response curve with time (CR_T) = (EC_{50} at time t/EC_{50} at $t = 0$) was used to correct the concentration-ratio obtained for antagonist (CR) = (EC_{50} in the presence of antagonist/EC_{50} in the absence of antagonist) according to the formula: adjusted $CR = CR/CR_T$ (Zhang and Dyer, 1990).

These adjusted concentration ratios were used to calculate the dissociation constant (K_B) of prazosin. The prazosin pA_2 value was calculated by Schild plot analysis as described by Furchgott (1972). Under equilibrium conditions:

$$pA_2 = -\log K_B$$

where pA_2 is the negative logarithm of the concentration of the antagonist required to give a concentration ratio of 2, and K_B is a quantitative measure of the dissociation of the receptor-antagonist complex. An alternate method (Furchgott, 1972) was used to determine the apparent dissociation constant (K_B) for WB4101. The apparent dissociation constant K_B for the antagonist was calculated by using the following formula:

$$\text{Apparent } K_B = [\text{antagonist}]/(CR - 1)$$

where [antagonist] represents the molar concentration of the antagonist and the concentration ratio (CR) is calculated as described above.

2.3. Determination of the dissociation constant (K_A) for phenylephrine

K_A values for phenylephrine were determined as described by Furchgott and Bursztyn (1967) and as previously carried out in our laboratory (Isla and Dyer, 1990a). A concentration-response relationship for phenylephrine was initially obtained. Then, dibenamine, in concentrations of 0.75–0.8 μM, was incubated with the tissue for 20 min to inactivate a fraction of the receptors. The tissue bath fluid was then changed 6 times with fresh Krebs' solution over 30 min. A second concentration-response relationship for phenylephrine was obtained.

2.4. Determination of α_1-adrenoceptor subtypes in porcine uterine artery

Uterine arterial rings were studied under conditions described above, i.e. with propranolol and inhibitors of uptake, monoamine oxidase and catechol-*o*-methyl transferase being used to block sites of loss before adding noradrenaline. Nifedipine, a dihydropyridine-type Ca^{2+} channel antagonist, was used to study the importance of Ca^{2+} influx in agonist-induced contraction (Minneman, 1988; Han et al., 1990; Isla and Dyer, 1990b). To one tissue bath, nifedipine (1.0 μM) was added for 20 min and a second concentration-response relationship to noradrenaline was generated in the presence of nifedipine. A paired 'time control' tissue was run which received no nifedipine. Experiments in which nifedipine was used were carried out in a darkened room. To another tissue bath, the irreversible alkylating agent chloroethylclonidine (50 μM) was added for 30 min. The tissue bath was then washed 5–6 times with Krebs' solution over 40 min. A second concentration-response relationship to noradrenaline was then obtained. A paired 'time control' tissue in each experiment was used to correct for time-dependent changes in tissue sensitivity.

2.5. Statistical analysis

Analysis of variance was used to analyze the data obtained for the determination of the dissociation constant for prazosin, phenylephrine and WB4101. The experiments involving nifedipine and chloroethylclonidine for the subclassification of α_1-adrenoceptor subtypes were analyzed by using an unpaired Student's t-test.

2.6. Drugs

The following drugs were used: phenylephrine HCl USP (Winthrop Laboratories); prazosin HCl and nifedipine (Pfizer); iproniazid phosphate (Hoffman-La Roche); tropolone (Aldrich); propranolol HCl (Ayrest Laboratories); cocaine, indomethacin, (−)-noradrenaline and corticosterone 21-acetate (Sigma); dibenamine HCl (Smith, Kline and French); chloroethylclonidine (Research Biochemicals); WB4101 (Dr. W.L. Nelson, University of Washington). All the drugs were dissolved in saline except corticosterone, indomethacin, dibenamine and nifedipine which were dissolved in ethanol.

3. Results

Phenylephrine produced concentration-dependent contractions of porcine uterine artery from the luteal phase, follicular phase and early pregnancy. Prazosin, an α_1-adrenoceptor antagonist, competitively inhibited responses to phenylephrine of uterine artery from the follicular phase (Fig. 1) as well as the luteal phase and early pregnancy (data not presented). A Schild plot for prazosin vs. phenylephrine yielded a straight line for arteries obtained in the follicular phase (Fig. 2) and in the luteal phase and in early pregnancy (data not presented) with slopes not significantly different from unity. The pA_2 values are presented in Table 1.

In the determination of the K_A for phenylephrine on

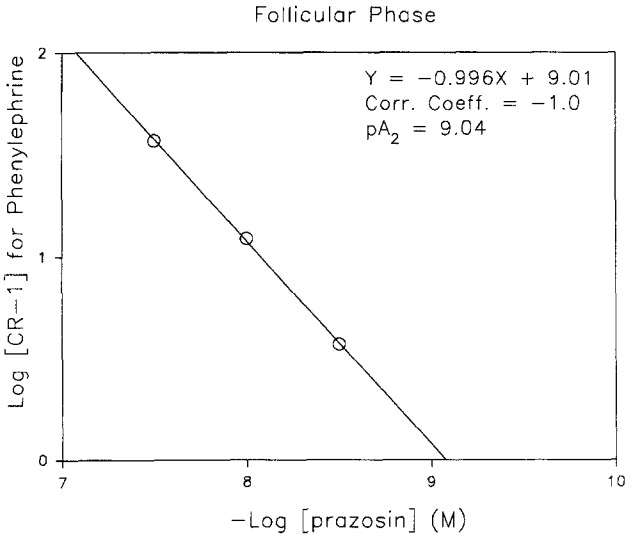

Fig. 2. Schild plot for determination of the pA_2 for prazosin when tested against phenylephrine on isolated rings of porcine uterine artery obtained in the follicular phase. Each point represents the mean of six experiments. The intercept on the abscissa gives the pA_2 value. The slope of the fitted regression line is shown in the figure. See Materials and methods for details.

porcine uterine artery, dibenamine reduced the response to phenylephrine about 40–60% in the luteal phase, 25–75% in the follicular phase and 50–75% in early pregnancy. The dissociation constants (K_A) for phenylephrine are presented in Table 1. The K_A values are not significantly different from each other ($P > 0.05$).

Noradrenaline produced concentration-dependent contractions of isolated uterine artery obtained during the luteal and follicular phases, and early pregnancy. The putative selective α_{1A}-adrenoceptor antagonist WB4101 (10 nM) inhibited responses to noradrenaline in arteries obtained during the follicular phase (Fig. 3), luteal phase and early pregnancy (data not presented). The dissociation constants (K_B) were not significantly different from each other ($P > 0.05$) and are presented in Table 2.

While the K_A (phenylephrine) and K_B (prazosin and WB4101) determinations were not different between the three groups (luteal, follicular and early pregnancy), there was a significant difference in their maximal response to phenylephrine (Table 3). The tension developed was greatest in uterine arteries obtained during early pregnancy,

Table 1
Dissociation constants for phenylephrine (K_A) and prazosin (K_B)

Stage	(n)	K_A	(n)	pA_2	K_B
Luteal phase	(3)	6.5 ± 1.8 μM [a]	(6)	8.98 [a]	1.04 nM
Follicular phase	(3)	3.7 ± 0.7 μM [a]	(6)	9.04 [a]	0.91 nM
Early pregnant	(3)	4.4 ± 1.7 μM [a]	(6)	9.10 [a]	0.79 nM

pA_2 values were determined by Schild plot analysis with phenylephrine as the agonist and prazosin as the antagonist. K_A values were determined as described in Materials and methods.
[a] Superscript not different from each other ($P > 0.05$).

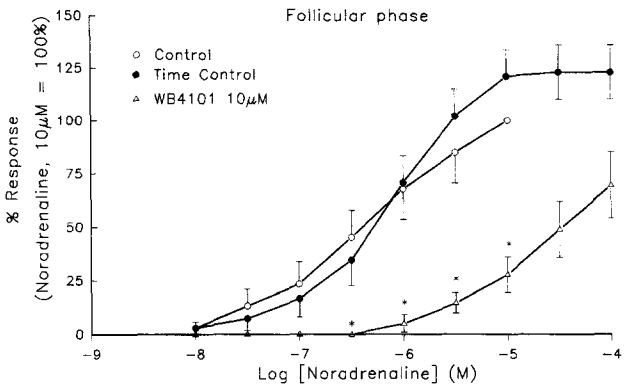

Fig. 3. Effect of WB4101 (10 μM) on noradrenaline-induced contractions of isolated porcine uterine artery from the follicular phase. Each point represents the mean ± S.E. of experiments from 5 animals. Points with asterisk (*) are significantly different from the time control ($P < 0.05$).

followed by arteries from the follicular phase and then the luteal phase.

The selective irreversible α_{1B}-adrenoceptor antagonist chloroethylclonidine (50 μM) displaced the noradrenaline concentration-response relationship curve to the right and also significantly depressed the maximum response to noradrenaline of uterine arteries obtained in the follicular phase (Fig. 4), in the luteal phase (data not presented) and in early pregnancy (Fig. 5). Chloroethylclonidine did not contract any of the tissues.

Another drug used to characterize α_1-adrenoceptors was nifedipine. Nifedipine reduced the maximum contractile response to noradrenaline, 41.5% in the follicular phase (Fig. 6), 26.1% in the luteal phase and 16.4% in early pregnancy.

Table 2
Dissociation constants for WB4101

Stage	K_B
Luteal phase	1.17 nM [a]
Follicular phase	0.17 nM [a]
Early pregnant	0.54 nM [a]

K_B = [antagonist]/(CR-1), where CR is the concentration ratio at the EC_{50} for noradrenaline alone and in the presence of WB4101 (see Materials and methods). Each K_B is the mean value for 5 experiments.
[a] Superscript not different from each other ($P > 0.05$).

Table 3
Comparison of maximal contractility to phenylephrine (100 μM)

Stage	Tension developed (g)	
	Mean	± S.E.
Luteal phase	8.73	± 1.53 [a]
Follicular phase	11.13	± 1.36
Early pregnant	17.05	± 2.50

Mean maximal contractility (± S.E.) of six 'time control' (Fig. 1) tissues.
[a] Superscript indicates values significantly different ($P < 0.05$) from early pregnant. None of the other mean values were significantly different from each other.

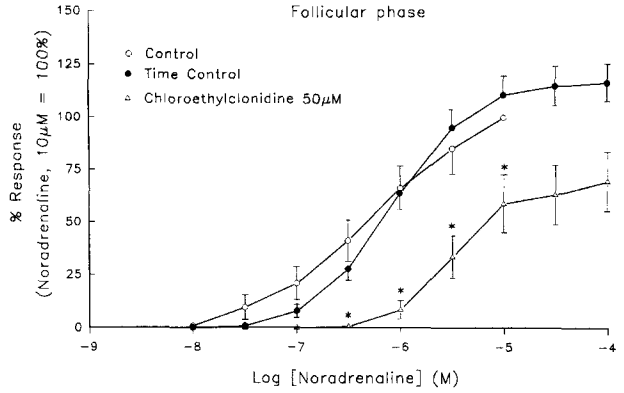

Fig. 4. Effect of chloroethylclonidine (50 μM) for 30 min followed by 40 min washout on noradrenaline-induced contractions of isolated porcine uterine artery obtained from the follicular phase. Each point represents the mean ± S.E. of experiments form 5 animals. Points with asterisk (*) are significantly different from the time control ($P < 0.05$).

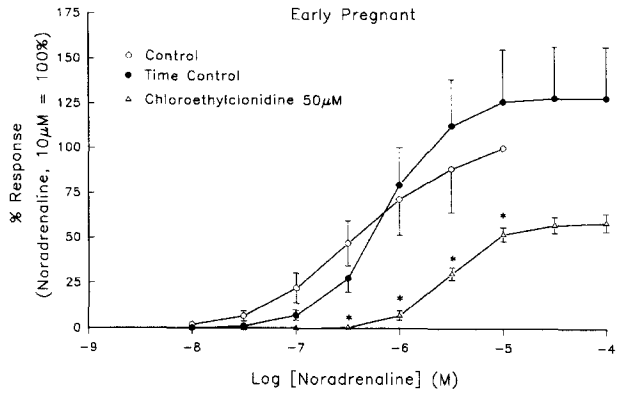

Fig. 5. Effect of chloroethylclonidine (50 μM) for 30 min followed by 40 min wash on noradrenaline-induced contractions of isolated porcine uterine artery obtained from early pregnant animals. Each point represents the mean ± S.E. of experiments from 5 animals. Points with asterisk (*) are significantly different from the time control ($P < 0.05$).

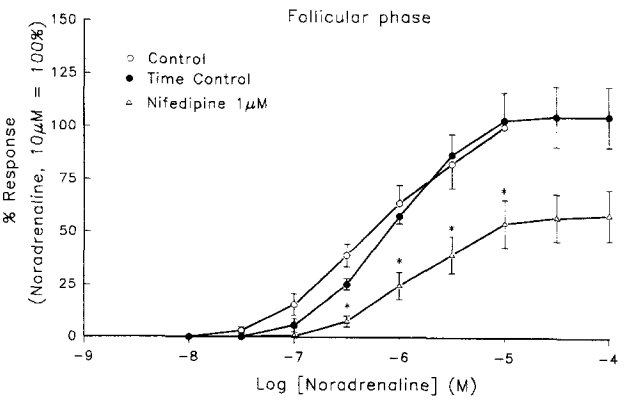

Fig. 6. Effect of nifedipine (1 μM) on noradrenaline-induced contractions of isolated porcine uterine artery in the follicular phase. Each point represents the mean ± S.E. of experiments from 4 animals. Points with asterisk (*) are significantly different from the time control ($P < 0.05$).

Ethanol (35.4 mM), the solvent used for nifedipine, dibenamine and indomethacin, had no effect on noradrenaline-induced contractions in tissues from the follicular phase ($n = 5$), luteal phase ($n = 3$) or early pregnancy ($n = 5$) (data not presented).

4. Discussion

In the present study, α_1-adrenoceptors in isolated porcine uterine artery were characterized based on the dissociation constant (K_A) for phenylephrine and the dissociation constant (K_B) of the competitive antagonist prazosin. Contractions to phenylephrine were competitively antagonized by prazosin. The pA_2 values for prazosin were 8.98, 9.04 and 9.10 for uterine arteries obtained from animals in the luteal, follicular or in early pregnancy, respectively. This indicates that prazosin has a high affinity for the α_1-adrenoceptor. The pA_2 values obtained in this study are very similar to that obtained in the non-pregnant human uterine artery (Fontes Ribeiro and Macedo, 1986) and in ovine uterine arteries from late pregnancy (Isla and Dyer, 1990a). The similarity of the pA_2 values suggests that uterine artery α_1-adrenoceptor affinity does not change during the estrous cycle and during early pregnancy.

The K_A values for phenylephrine in the luteal and follicular phases and in early pregnancy (Table 1) were not significantly different from each other. These values are similar to the K_A value of 2.5 μM for phenylephrine found in the ovine uterine artery from late pregnancy (Isla and Dyer, 1990a).

The similarity in K_A and K_B values cited above is consistent with the notion that α_1-adrenoceptor affinity is not different between the three groups of arteries. However, the maximal contractility to the α_1-adrenoceptor agonist, phenylephrine, is different (Table 3). It may be that muscle mass or tissue-receptor coupling mechanisms are enhanced during pregnancy and this then explains, in part, these observations. Additional experiments will be required to explain these findings.

Radioligand binding and functional studies have supported the existence of two pharmacologically distinct α_1-adrenoceptor subtypes, (Morrow and Creese, 1986; Han et al., 1987a,b) in vascular as well as in non-vascular tissues. We used WB4101, nifedipine and chloroethylclonidine as pharmacological tools to ascertain if the porcine uterine artery possessed the α_{1A}- and/or α_{1B}-adrenoceptor subtype. The results presented here suggest the presence of both α_{1A}- and α_{1B}-adrenoceptor subtypes in the porcine uterine artery. The high affinity (0.17–1.17 nM, K_B values) for the competitive antagonist WB4101, in the luteal phase, follicular phase and in early pregnancy suggests that the contractile responses to noradrenaline are mediated by α_{1A}-adrenoceptors. Similar K_B values and a high affinity for WB4101 (Minneman, 1988) would be consistent with characterization of the porcine uterine artery as possessing α_{1A}-adrenoceptors in the luteal and follicular phases and in early pregnancy.

Chloroethylclonidine preferentially and irreversibly inactivates the α_{1B}-adrenoceptor subtype (Han et al., 1987a,b; Minneman et al., 1988). Chloroethylclonidine nearly abolished contractions to noradrenaline in rat aorta (Han et al., 1990). In our experiments, chloroethylclonidine pretreatment reduced the maximum response of the porcine uterine artery to noradrenaline by 30–44% in the luteal and follicular phases and in early pregnancy, but these inhibitions were not significantly different from each other. These results suggest the presence of α_{1B}-adrenoceptor subtypes. Our results with WB4101 and chloroethylclonidine suggest the presence of both α_{1A}- and α_{1B}-adrenoceptor subtypes and are consistent with those found in rat mesenteric artery and portal vein (Han et al., 1987a). In our study, we did not observe a contractile effect to chloroethylclonidine, although the maximum response to noradrenaline was reduced. A similar observation has been made in rat vena cava (Sayet et al., 1993) where chloroethylclonidine did not cause a contraction yet reduced the maximum response to noradrenaline. However, chloroethylclonidine initiated a contraction of the rabbit ear, renal and mesenteric arteries (Oriowo et al., 1992) and the rat thoracic aorta (LeClerc et al., 1980; Oriowo and Bevan, 1990). The maximum response to noradrenaline in rabbit arteries (Oriowo et al., 1992) and in rat and rabbit aorta (Tian et al., 1990) was not altered by chloroethylclonidine. Responses to noradrenaline were shifted to the right by chloroethylclonidine with no reduction in the maximum response of aortas from rat, dog, rabbit and guinea-pig (Oriowo and Ruffolo, 1992). In this regard, the porcine uterine artery differs from most studies on blood vessels in that chloroethylclonidine reduced the maximum response to noradrenaline.

It has been hypothesized that evaluation of the dependence on extracellular Ca^{2+} for the α-adrenergic response might provide a means for differentiation between α_{1A}- and α_{1B}-adrenoceptor subtypes (Minneman, 1988). In our study, nifedipine significantly reduced (16.4–41.5%) the maximum response to noradrenaline and also shifted the concentration response curve for noradrenaline to the right. This suggests that the contractile responses were not independent of the presence of extracellular Ca^{2+}. A similar observation has been made in vas deferens which contains both α_{1A}- and α_{1B}-adrenoceptor subtypes (Han et al., 1987b). However, the magnitude of inhibition in our study was much less than that observed in the vas deferens. Since the response to noradrenaline was reduced but not abolished after nifedipine treatment, we suggest that both extracellular as well as intracellular Ca^{2+} are involved in mediating the contractile response. The ability of chloroethylclonidine and WB4101 to block and nifedipine to partially block contractions to noradrenaline suggests that the porcine uterine artery possesses both α_{1A}- and α_{1B}-adrenoceptors. However, recent suggestions by Oriowo and Ruffolo (1992) indicate that the combined use of chloroethylclonidine, WB4101 and Ca^{2+} channel blockers may be inadequate as pharmacologic tools for the subclassification of α_1-adrenoceptors in mammalian blood vessels.

In summary, our data indicate that α_1-adrenoceptor affinity of the porcine uterine artery does not change during the estrous cycle and in early pregnancy. In addition, we have also presented evidence suggesting that both α_{1A}- and α_{1B}-adrenoceptor subtypes exist in the porcine uterine artery.

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