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Porcine uterine artery: Pharmacological characterization of α-adrenergic receptors, and norepinephrine disposition

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Rita S. Nair

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CHAPTER 1. REVIEW OF LITERATURE

The uterine artery is a major source of blood supply to the uterus. The other two arteries supplying the uterus are the ovarian and vaginal arteries. The uterine artery arises from the umbilical artery, which originates from the internal iliac artery [28].

The walls of the arteries supplying the uterus are composed of three layers: the tunica intima, the tunica media, and the tunica adventitia [71]. The tunica intima is the innermost layer, consisting of a single layer of endothelial cells attached to the basement membrane. Endothelial cells are flat and elongated cells with a thickness of about 0.1-0.2 μm. The endothelial cells are held together by junctional complexes. The tunica media is the middle layer of the vascular wall, and is composed largely of spindle shaped smooth muscle cells with a fine collagen and elastic network between them. A thick internal elastic lamina separates the tunica media and tunica intima. This internal elastic lamina is about 3μm in thickness and is fenestrated, which facilitates metabolic and diffusion processes in order to establish a membranous contact with smooth muscle cells of the tunica media. Longitudinally arranged smooth muscle cells are present in the region of the internal elastic lamina. Tunica adventitia, the outermost layer, is composed of dense fibroelastic tissue without smooth muscle cells. The adventitia gives rigidity to the vascular wall and connects blood vessels to

the surrounding tissues [71]. Adventitia is the site where vasomotor neurons course before terminating on the vascular smooth muscle cells.

The adrenergic innervation of most blood vessels is not uniform in density but is limited to the adventitiomedial junction as a continuous plexus surrounding the smooth muscle of the media [9]. This is supported by the results obtained in the rabbit saphenous artery, where the distribution of adrenergic plexus is not uniform along the length of the artery [7]. Thus, variation in the distribution of adrenergic innervation along the artery might lead to differences in the responses to sympathetic stimulation.

Blood flow to the reproductive tract is under the influence of sympathetic vasoconstrictor nerves [48]. The main sympathetic perivascular plexus surrounding the uterine arterial vessels are post ganglioinic fibers derived from the abdominal aortic plexus via the iliac plexus. The uterine artery receives additional sympathetic innervation from the utero-vaginal plexus [78]. Vasoconstriction mediated by α-adrenergic receptors predominates in the vessels of the uterus and ovaries. α-Adrenergic agonists such as phenylephrine, and norepinephrine are effective constrictors of human [17], ovine [39], and porcine [82] uterine vasculature.

The estrous cycle in the pig lasts approximately 21 days and is divided into the follicular phase and the luteal phase [80]. During this period blood flow to the uterus fluctuates, and this has been correlated with the ratio of estrogen and progesterone in the systemic blood [22]. Similar observations have been made in the ewe [31] and cow [21]. Estrogen has been reported to increase blood flow to the porcine uterus. On the other hand, progesterone has been reported to antagonize the effect of estrogen [69]. Ford et al. indicated that during the estrous cycle of gilts

the steroid hormone progesterone of endogenous or exogenous origin increases the number of α_1-adrenergic receptor number on uterine smooth muscle cells [23]. The phasic contractility of pig uterine artery has also been reported to be influenced by estrogen and progesterone. Uterine arterial contractility in gilts was higher during the luteal phase (high progesterone / low estrogen) of the estrous cycle and lower during the follicular phase (low progesterone / high estrogen) [23]. Furthermore, McKercher et al. [52] observed a reduction in norepinephrine content of uterine periarterial sympathetic nerves in estrogen treated rats. It has been suggested that estrogen decreases and progesterone increases the function of the periarterial sympathetic nerves.

Responsiveness of uterine arteries to adrenergic receptor agonists such as phenylephrine and norepinephrine has been reported to be different between nonpregnant and pregnant gilts [23]. A number of studies has indicated that pregnancy is associated with specific alterations of systemic vascular reactivity [13] & [14]. Ford et al. [23] reported that contractile responses of uterine artery to adrenergic receptor agonists is different during the estrous cycle in gilts. A variation in the adrenergic innervation in the rat uterus during the estrous cycle has been reported by Adham and Schenk [1]. Also there have been reports indicating a reduction in the adrenergic fibers in the dog [74] uterus by the end of the pregnancy. A similar reduction of adrenergic nerve terminals has been reported in pregnant guinea pig [79] and rabbit [72] uterus.

During the 114days gestation in the pig, the uterine arterial diameter has been reported to increase from $4.5\pm.6$ mm on day 0 to $9\pm.1$ mm on day 110 [32]. This change in arterial diameter has been attributed to a decline in the ratio of collagen to elastin throughout gestation. Similar results have been obtained in the guinea pig

where an increased diameter of arteries supplying the uterine tissues during pregnancy was correlated to changes in the nonmuscular element of the arterial wall and this effect was mediated by estrogen [55]. Collagen and elastin make up over one half of the dry weight of the arterial wall [20].

The neurotransmitter norepinephrine is synthesized, stored in and released from adrenergic nerve terminals. Norepinephrine is synthesized from its precursor, tyrosine, which is generally available in body fluids and is converted in the neuron to 3,4-dihydroxyphenylalanine (dopa) by tyrosine hydroxylase. Tyrosine hydroxylase, an important regulatory enzyme in the biosynthesis of norepinephrine, is present in the nerve axoplasm. Dopa decarboxylase, a nonspecific enzyme present in the cytosol, then converts dopa to dopamine. Dopamine is then converted to norepinephrine by dopamine β-hydroxylase, an enzyme closely associated with the norepinephrine storage granules. Norepinephrine is stored in a large number of dense core vesicles known as varicosities [50].

Upon nerve stimulation norepinephrine is released by exocytosis into the synaptic cleft. The released norepinephrine can then reach adrenergic receptor by diffusion and thereby produce its effect. Norepinephrine induces contraction of smooth muscle by interacting with membrane bound α-adrenergic receptors. After the release of norepinephrine from the terminal into the synaptic cleft, it is inactivated by specialized transport systems that mediate either reuptake of the released norepinephrine into the nerve terminals (neuronal uptake or uptake-1), or uptake into the non-neuronal sites (extraneuronal uptake or uptake-2). The two metabolic enzymes catechol-0-methyltransferase (COMT) and monoamine oxidase (MAO) also play an important role in the disposition of norepinephrine. Neuronal uptake involves the transport of

norepinephrine from the extracellular fluid across the neuronal membrane. On the other hand, extraneuronal uptake involves the influx of norepinephrine across cellular membrane other than those of neurons [10]. thus, the above mentioned processes (i. e., uptake-1, uptake-2, and metabolizing enzymes MAO & COMT) play a principal role in terminating the action of norepinephrine.

The two uptake processes, i.e., uptake-1 and uptake-2, involved in transporting norepinephrine differ in terms of their sodium dependency. Neuronal uptake is an active process and is absolutely dependent on the presence of sodium and chloride. The carrier involved in transporting norepinephrine across the neuronal membrane has three binding sites, one for sodium, one for chloride, and one for the norepinephrine. This carrier is mobile when all three binding sites are free but the binding of sodium to the carrier makes it immobile. The carrier regains its mobility as soon as chloride and norepinephrine binds to it [87]. The second uptake process (uptake-2) or extraneuronal uptake is not dependent on the presence of sodium and chloride [86].

Monoamine oxidase is a flavoprotein, which is localized in the mitochondria. It has been reported to be present in sympathetic neurons as well as in other adjacent cells [92]. Whereas, COMT has been reported to be present predominantly in the smooth muscle cells [90]. This differential localization of MAO and COMT is supported by experiments in which after sympathetic denervation the COMT activity of various tissues was unchanged. Monoamine oxidase activity was reduced but not abolished by sympathetic denervation [40].

Raab and Gigee [68], Nickerson and colleagues [62], were the first to demonstrate an increase in catecholamine content of cat, dog, and rat hearts after the administration of large doses of epinephrine and norepinephrine. Strombland and

Nickerson [83] found in their experiments that the rat heart and salivary gland could accumulate norepinephrine or epinephrine after *in vivo* or *in vitro* administration of catecholamines. From these results they concluded that this uptake by tissues, which is related to the sympathetic nerve ending (neuronal uptake), is a major factor in terminating the effect of catecholamines. Iversen [41], while working on isolated rat heart, suggested that catecholamines are taken up not only in neuronal cells but also by extraneuronal cells e.g., myocardial cells of the heart. When catecholamine concentration is low, there is no accumulation of norepinephrine inside the extraneuronal cells as it is metabolized by the enzymes MAO and COMT. Thus, deamination and O-methylation of catecholamines caused by MAO and COMT, respectively, are subsequent to uptake processes [86].

The route of disposition of catecholamines (norepinephrine) varies from vessel to vessel and depends on the width of the synaptic cleft as well as on the thickness of the vessel [10]. The nerve-muscle distance influences the concentration of the transmitter which can reach the postsynaptic receptor, and the different cell layers of the blood vessel wall [8]. In smaller blood vessels the nerve-muscle distance is small; hence the effect of diffusion is negligible causing the transmitter concentration to be localized within the synaptic cleft. This leads to a rapid contractile effect since the α-adrenergic receptors are located at the postsynaptic membrane. Because of functional coupling between the smooth muscle cells the spread of the contractile response occurs by myogenic propagation [6]. In larger vessels, α-adrenergic receptors are present throughout the blood vessel wall and the synaptic cleft is usually wider. Thus, transmitter (norepinephrine) will diffuse throughout the surrounding tunica media, resulting in a slow contractile response [6] . In vessels of intermediate size,

nerve-muscle distance is neither too small nor too large. In vessels of intermediate size, the contractile response is a combination of fast response (due to the high concentration of norepinephrine at the postsynaptic membrane) followed by a slow contractile response (due to the diffusion of norepinephrine to α-adrenergic receptors throughout the wall) [6].

The inhibition of neuronal uptake by cocaine and other pharmacological agents [87] is used to analyze the importance of uptake-1 in the disposition of exogenous and endogenous norepinephrine. Similarly, pharmacological blockade of extraneuronal uptake by corticosterone [86] and blockade of intracellular enzymes by tropolone (COMT inhibitor) and iproniazid (MAO inhibitor) [29] are used to assess the role of uptake-2, MAO & COMT in transmitter disposition.

Classification of Adrenergic Receptors

The concept of specific receptors was first introduced by Langley [47] who introduced the term receptive substance and proposed that the receptive substance receives the stimuli from drugs which is then transferred to the effector organs.

Ahlquist [3] proposed a division of adrenergic receptors into two major types: α- and β-adrenergic receptors. Studies have shown that there are two types of α-adrenergic receptors, designated α_1- and α_2-adrenergic receptors [46]. Further studies have shown that the α_1-, α_2- and β-adrenergic receptors can each be further subdivided as shown in Figure 1.1. [12].

Langer [46] proposed a classification of α_1- and α_2-adrenergic receptors. This classification was initially suggested on an anatomical basis to differentiate postjunctional α-adrenergic receptors (α_1) and prejunctional release modulating receptors (α_2).

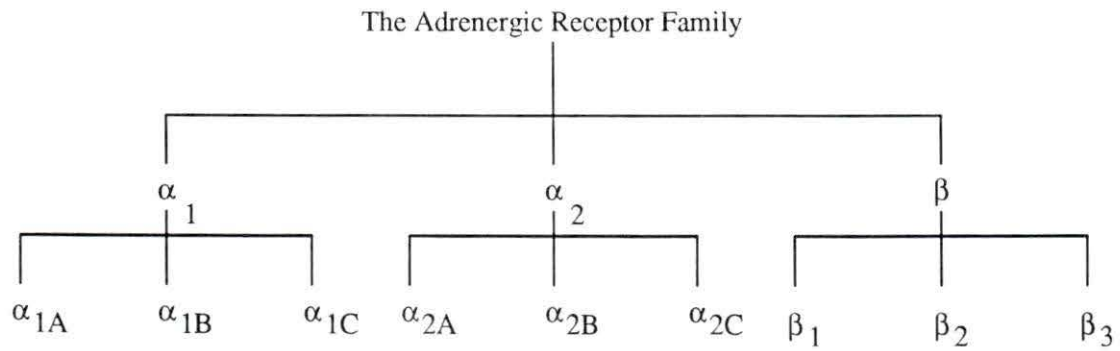


Figure 1.1: Adrenergic Receptor Family

α-adrenergic receptors (α_1) and prejunctional release modulating receptors (α_2). Berthelsen and Pettinger [5] proposed a functional classification of α-adrenergic receptors, into α_1- and α_2- adrenergic receptors rather than one based on anatomical classification. Because anatomical classification was not relevant in all tissues, Drew and Whiting [15] indicated that neither anatomical nor functional classification can be used as a basis to classify adrenergic receptors.

Now α-adrenergic receptors are classified based on their affinities for agonists and antagonists. Differences in antagonist affinities are usually used as a criterion to classify receptors. Alpha adrenergic receptors that are activated by either methoxamine or phenylephrine and blocked in a competitive manner by low concentration of prazosin are classified as α_1-adrenergic receptors [73] and α-adrenergic receptors that are activated by clonidine or medetomidine and blocked by low concentration of yohimbine are classified as α_2-adrenergic receptors.

α_1-Adrenergic receptor subtypes

There is increasing evidence from radioligand and functional studies that α_1-adrenergic receptors can be further subdivided into two pharmacologically distinct

WB4101 were resolved into two binding site models when [^{3}H] prazosin was used as a radioligand [4]. These results were confirmed by Morrow and Creese [56] in the rat cerebral cortex. They formally proposed the definition of α_{1A}- and α_{1B}- adrenergic receptor subtypes. They indicated that [^{3}H] prazosin had similar or equal affinities for the two binding sites but WB4101 and phentolamine were 20-40 times more potent at the α_{1A}-adrenergic receptor site as compare to the α_{1B}-adrenergic receptor site. They proposed that binding sites with subnanomolar affinity for WB4101 are α_{1A}-adrenergic receptor subtypes whereas those with low affinity for WB4101 are α_{1B}-adrenergic receptor subtypes.

Chlorethylclonidine (CEC) is an irreversible alkylating derivative of clonidine [49]. Chlorethylclonidine has differential effectiveness at α_1-adrenergic receptors subtypes. It has been proposed as a pharmacological tool to distinguish between the two subtypes of α_1-adrenergic receptors. Chlorethylclonidine distinguishes the two subtypes of α-adrenergic receptors in rat liver, spleen, and vas(ductus) deferens [34]. Johnson and Minneman [42] demonstrated that CEC inactivated only approximately 50% of the α_1-adrenergic receptors in the rat cerebral cortex but did not inactivate any α_1-adrenergic receptors in rat hippocampus . They confirmed that CEC could discriminate between the two α_1-adrenergic subtypes. Their results suggested that there are at least two pharmacologically distinct subtypes of α_1-adrenergic receptors in mammalian tissues, only one of which is inactivated by the alkylating agent CEC. Thus, CEC sensitive α_1-adrenergic receptors are designated as α_{1B}-adrenergic receptor subtypes and CEC insensitive receptors as α_{1A}-adrenergic receptor subtypes.

Calcium ions play an important role in the regulation of many cellular processes, including vesicular exocytosis and muscle contraction. It is generally accepted that

α_1-adrenergic receptors act by increasing formation of inositol (1,4,5) trisphosphate (IP_3) and diacylglycerol (DAG). Inositol 1,4,5-trisphosphate and diacylglycerol act as second messengers, the former mobilizing intracellular calcium and the latter activating protein kinase c [88]. α_1- Adrenergic receptor mediated contraction in smooth muscle is regulated by both intra- and extracellular calcium. Han et al. [33] indicated that the two α-adrenergic receptor subtypes might be linked to different intracellular processes. Thus, the hypothesis has been put forward that α_1-adrenergic receptors subtypes may be linked to different signal transduction pathways. The α_{1A}-adrenergic receptor subtype has been proposed to control influx of extracellular calcium into the cell through the voltage dependent calcium channels. However, the α_{1B}-adrenergic receptor subtype mediates hydrolysis of phosphatidylinositol bisphosphate (PIP_2) to inositol 1,4,5-trisphosphate and diacylglycerol. This differential involvement of extracellular calcium in the actions of α_{1A}- and α_{1B}-adrenergic receptors has been confirmed by Tsujimoto et al. [88] while studying phosphorylase activation in rat hepatocytes and rabbit aorta. A similar observation has been made by Han et al. [33]. Their results indicate that in rat spleen, which possess the α_{1B} -adrenergic receptor subtype, contractile responses were independent of the presence of extracellular calcium, whereas the rat vas deferens which contains both, the α_{1A}- and α_{1B}-adrenergic receptor subtype (40% and 60%, respectively), contractile responses were markedly reduced on removal of calcium. However, Klijn et al. [43] reported that the D1 clone of Madin darby canine kidney cell line (MDCK-D1), which is of the α_{1B}-adrenergic receptor subtype, mediates responses that are both sensitive to CEC and also dependent on extracellular calcium.

α_2-Adrenergic receptors subtypes

That the α_2-adrenergic receptor type does not represent one homogenous population of receptors has been indicated for many years [85]. A consistent pharmacological definition of α_2-adrenergic receptors subtypes has resulted mainly from radioligand binding studies. Based on the pharmacologically determined affinity to the α_2-adrenergic receptor antagonist yohimbine, it was suggested that there could be two different populations of α_2-adrenergic receptors. However, these results were obtained from two different species: rodent *vs* nonrodent [16]. Waterfall et al. [91], while studying rat and rabbit vas deferens, indicated that the affinity of rauwolscine, yohimbine, and substituted benzoquinolizines was almost similar at the prejunctional α_2-adrenergic receptors in the rat vas deferens. However, the affinities of yohimbine and rauwolscine were approximately 100-fold greater than those of benzoquinolizines in the rabbit vas deferens. This supports the observation of α_2-adrenergic receptor heterogenity between species.

While receptor heterogenity between species is important, receptor subtypes are usually characterized based on their heterogenity within the same species or within a single tissue. Bylund [11] suggested that α_2-adrenergic receptor heterogenity does exist in tissues from a single species. His findings indicated that several α_2-adrenergic receptor antagonists, including prazosin and oxymetazoline, had significantly different affinities in inhibiting [^{3}H] yohimbine binding in various regions of both rat [11] and human brain [66]. Thus, subclassification of α_2-adrenergic receptors was proposed based on the relative potency of prazosin [11]. Receptors having a low affinity for prazosin, as demonstrated in human platelet, were classified as α_{2A}-adrenergic receptors. Whereas, receptors having a relatively higher affinity for prazosin, as in

neonatal rat lung and rat kidney, were proposed to be α_{2B}-adrenergic receptors [11]. A similar definition of α_{2A}- and α_{2B}-adrenergic receptor subtypes was proposed independently by other authors [60]. To-date there have been numerous reports confirming α_2-adrenergic receptor heterogenity in various tissues. For instance, α_2-adrenergic receptors in rabbit spleen were shown to display a pharmacology consistent with α_{2A}-adrenergic receptor subtypes while in the rat kidney α_2-adrenergic receptor subtypes have been reported to be of the α_{2B}-adrenergic receptor subtype [53]. Furthermore, rat brain has been reported to possess both α_{2A}- and α_{2B}-adrenergic receptor subtypes [11].

A third α_2-adrenergic receptor subtype has been identified in opossum kidney (OK) derived cell line. This receptor subtype has a unique pharmacological profile which is closer to the α_{2B}- than to the α_{2A} -adrenergic receptor subtype. The adrenergic receptor subtype which has been identified in OK derived cell line has high affinity for prazosin, but the K_i (affinity) ratio of prazosin to yohimbine is intermediate between that of α_{2A}- and α_{2B}-adrenergic receptor subtype. However, more extensive characterization in opossum kidney cell has indicated this receptor subtype to be different from α_{2A}-and α_{2B}-adrenergic subtypes. Thus, it has been proposed that OK cells represent a third α_2-adrenergic receptor subtype which has been designated as an α_{2C}-adrenergic receptor subtype [58].

The mechanism of action for α_2-adrenergic receptors is the inhibition of adenylyl cyclase. Unlike α_1-adrenergic receptors subtypes all three α_2-adrenergic receptor subtypes i.e., α_{2A}, α_{2B}, and α_{2C} have been shown to inhibit adenylyl cyclase [58].

Several ligands have been shown to possess a good degree of selectivity for one or the other of these α_2-adrenergic receptor subtypes. Various pharmacological

agents have been used to subclassify α_2-adrenergic receptor subtypes. Benoxathian, oxymetazoline and WB4101 have been proposed to be relatively selective for α_{2A}-adrenergic receptors. Prazosin, chlorpromazine and imiloxan are relatively selective for α_{2B}-adrenergic receptors [53], whereas BAM1303 is relatively selective for α_{2C}-adrenergic receptors [12]. Although the above mentioned drugs are relatively specific for one or the other α_2-adrenergic receptor subtypes, to-date there are no drugs which are exclusively selective for a given α_2-adrenergic receptor subtype.

Classification of β-adrenergic receptors

The concept of distinct α- and β-adrenergic receptors was supported by Powell and Slater [67]. With the discovery of potent and selective β-adrenergic receptor agonists and antagonists, it became clear that β-adrenergic receptors were not homogeneous [45]. Classification of β-adrenergic receptors into β_1- and β_2-adrenergic receptor subtype was proposed by Lands et al. [45]. The subclassification of β-adrenergic receptors into β_1- and β_2-adrenergic receptor subtypes is now firmly established. Now there is a strong evidence for an atypical β- adrenergic receptor in several tissues. This atypical β- adrenergic receptor has been designated as a β_3-adrenergic receptor subtype [84].

Unlike α_1-adrenergic receptor subtypes, the β-adrenergic receptor subtypes do not use different signal transduction mechanism. All β-adrenergic receptor subtypes appear to be directly linked to the activation of adenylyl cyclase [81].

CHAPTER 2. STATEMENT OF PROBLEM

α_1- Adrenergic receptors, located in several tissues throughout the body, mediate many responses to catecholamines. An important α_1-adrenergic response is contraction of vascular smooth muscle. α_1-Adrenergic receptor mediated contraction of vascular smooth muscle plays an important role in controlling the peripheral circulation. Adrenergic receptor agonists such as phenylephrine and norepinephrine are effective constrictors of human, ovine, and porcine uterine vasculature.

Contractile responses of uterine arteries to adrenergic receptor agonist have been reported to be different between estrous cycle (nonpregnant day 13, nonpregnant day 19-21) and pregnant gilts (day 13). Furthermore, α-adrenergic receptor binding analysis, using [^{3}H] WB4101 as a ligand, has indicated a difference in binding between estrous cycle and early pregnant gilts. However, no detailed functional pharmacological characterization of uterine vasculature adrenergic receptors between nonpregnant and various stages of pregnancy in either gilts or sows has been reported.

The purpose of this study was as follows:

1) To pharmacologically characterize the α-adrenergic receptors of the isolated porcine uterine artery in the luteal and, follicular phase and in early pregnancy and;

2)To ascertain the functionality of various disposition mechanisms for norepinephrine in the arterial wall by determining the magnitude of norepinephrine po-

tentiation in response to inhibition of uptake-1, uptake-2, monoamine oxidase, and catechol-o-methyltransferase.

CHAPTER 3. MATERIALS AND METHODS

General Considerations

Specimens of uterine arteries were obtained from pigs in estrous cycle (luteal phase and follicular phase), and in early pregnancy (approx. 40-45 days). The crown-rump measurement [19] was done in order to determine the day of pregnancy. The crown-rump measurement is the distance between the highest point of the head and the base of the tail. The difference between the luteal phase and follicular phase of the estrous cycle was made based on the presence (luteal phase) or the absence (follicular phase) of the corpus luteum. Specimens were collected from the Iowa Packing Plant in Des Moines. 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 0.03mM), indomethacin (10^{-6}M and aerated with air. The tissues were then transported to the laboratory. EDTA was added to suppress the oxidation of amines and indomethacin was added to inhibit prostaglandin synthesis.

The (middle) uterine artery supplying one uterine horn was separated from surrounding tissues. Tissues not used immediately were stored upto 24 hours at 4°C in Krebs solution which was continuously oxygenated with a (95:5) oxygen and carbon

dioxide mixture. Isolated arterial rings of about 5mm were prepared from the (middle) uterine artery before the first bifurcation. Two stainless steel hooks were passed through the vessel lumen [38]. One hook was attached to the the base of the bath and the other was attached to the Grass force transducer FT03 which was in turn connected to a Beckman R-611 8 channel chart recorder. Tissue contractions were recorded isometrically. The ring segments were suspended in 10ml isolated organ baths, maintained at 37°C, bathed with a Krebs solution and aerated continuously with a oxygen- carbon dioxide (95:5) mixture. The ring segments were initially equilibrated for 30 minutes under no tension. The tissues were then placed under, 4g tension for 30 minutes period [75]. The ring segments were then brought to a resting tension of 2g and were further equilibrated for 60 minutes with regular replacement of bath fluid at 15 minute intervals. Tissues were maintained at 2g tension throughout the experiment.

Determination of the Dissociation Constant (K_B)

Uterine ring segments from the same uterine artery were prepared and suspended in Krebs solution. Iproniazid (0.36 mM) was added to the bath for 60 minutes and then washed for 40 minute at 10 minute intervals. Cocaine (10^{-5}M), tropolone (10^{-5}M), propranolol (10^{-6}M), and corticosterone acetate (10^{-6}M), were added for 20 minutes and the concentration-response relationship to phenylephrine was obtained in the presence of these drugs. Iproniazid was added to block monoamine oxidase while cocaine and corticosterone were used to block uptake mechanisms. Tropolone was added to inhibit COMT and propranolol to block β-adrenergic receptors. A first concentration-response relationship to phenylephrine was obtained

following the cumulative addition of phenylephrine in approximately one-half log increments [89]. After completion of the first concentration-response relationship, the tissue baths were washed for 60 minutes at 15 minute intervals. Three different concentrations of prazosin (3 x 10^{-9}, 10^{-8}, and 3 x 10^{-8}M) were added to the tissue baths and were allowed to equilibrate for 60 minutes. 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 as a "time control", and was used to correct for any changes in tissue sensitivity during the course of the experiment.

The shift of the log concentration-response relationship to phenylephrine in the presence and in the absence of prazosin was determined at the EC_{50}. 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 time 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 $CR = CR/CR_T$

These adjusted concentration ratios were used to calculate the dissociation constant of prazosin. Prazosin dissociation constant was calculated by the method of the Schild as described by Furchgott [27]. From the plot of log(CR-1) against log [B], K_B was calculated by using the equation:

$log(CR - 1) = log[B] - logK_B$

where CR is the concentration ratio, which is the ratio of concentration of agonist giving an equal response in the presence and in the absence of antagonist, and [B] is the concentration of the antagonist. If the blockade is competitive, a plot of the

logarithm of (CR-1) against the negative logarithm of the molar concentration of antagonist should yield a straight line whose slope is - 1 and the intercept along the abscissa is the pA_2 value, which is equal to $-logK_B$. Under equilibrium conditions:

pA_2 = -logK_B

where pA_2 is the negative logarithm of the concentration of the antagonist required to give a dose ratio of 2, and K_B is a quantitative measure of the dissociation of the receptor-antagonist complex.

An alternate method was used to determine the apparent dissociation constant(K_B) for WB4101. Uterine ring segments were treated with inhibitors for uptake-l and uptake-2 as well as for MAO and COMT and a first concentration-response relationship to norepinephrine was obtained as described in section entitled "Determination of the Dissociation Constant". After completion of the first concentration-response relationship, the tissue baths were washed for 60 minutes at 15 minute intervals. In order to determine the apparent dissociation constant (K_B) for WB4101 a single concentration (10^{-8}M) of antagonist WB4101 was used. WB4101 (10^{-8}M) was added to the one tissue bath and was equilibrated with the tissue for 60 minutes. A tissue which received no antagonist was used as a "time control". A norepinephrine concentration-response relationship was then repeated in the presence of antagonist. The apparent dissociation constant K_B for the antagonist was calculated by using the following formula:

Apparent K_B =[antagonist]/(CR-1)

where [antagonist] represents the molar concentration of the antagonist and the concentration ratio (CR) is calculated as above (EC_{50} of the agonist in the presence of the antagonist divided by the control EC_{50}).

Determination of the Agonist Dissociation Constant (K_A) for Phenylephrine

Ring segments of uterine artery were prepared and suspended in Krebs solution as described in the section entitled "General Considerations". Ring segments were pretreated with iproniazid (0.36mM). Cocaine (10^{-5}M), corticosterone (10^{-6}M), tropolone (10^{-5}M), and propranolol (10^{-6}M) were added for 20 minutes before starting the concentration- response relationship to phenylephrine. A concentration-response relationship to phenylephrine was obtained by the cumulative addition of phenylephrine in approximately one-half log increments to the bath [89]. After completion of the first concentration-response relationship to phenylephrine the tissues were washed for 60 minutes at 15 minute intervals. Dibenamine in the following concentrations was used to inactivate a fraction of the receptors: (6×10^{-6}M) in the luteal phase, ($7.5\text{-}8.0 \times 10^{-7}$M) in the follicular phase and (7.5×10^{-7} - 1×10^{-6}M) in early pregnancy. Dibenamine was added to the tissue bath for a 20 minute period to inactivate the α-receptors. The tissue baths were then washed 5-6 times over 30 minutes. A second concentration-response to phenylephrine was then obtained. To obtain the K_A values, an analysis of the plotted concentration response data was made as described by Furchgott and Bursztyn [26]. Equieffective concentrations of phenylephrine before [A] and after [A$'$] partial receptor inactivation were obtained from the concentration-response curves and a plot of 1/A versus 1/A$'$ was generated. The slope of the regression line and the Y-intercept were used to calculate K_A using the equation:

$$1/[A] = (1-q)/qK_A + 1/q[A']$$

where q is the fraction of active receptors remaining after irreversible inactivation.

Hence,

$K_A = slope - 1/intercept$

Determination of α_1-Adrenergic Receptor Subtypes in Porcine Uterine Artery

Uterine ring segments from the same uterine artery were prepared and suspended in Krebs solution as described in the section entitled "General Considerations". In brief, ring segments were pretreated with iproniazid (0.36mM) for 60 minutes. The tissues were then washed for 4-5 times over 40 minutes. Cocaine (10^{-5}M), corticosterone (10^{-6}M), tropolone (10^{-5}M), and propranolol (10^{-6}M) were added to the tissue baths for 20 minutes before starting the initial concentration- response relationship to norepinephrine. To one tissue bath, nifedipine (10^{-6}M) was added for 20 minutes and a second concentration-response relationship to NE was generated in the presence of nifedipine. A paired "time control" was run which received no nifedipine. Experiments in which nifedipine was used were carried out in a darkened room. To the another tissue bath the irreversible alkylating agent chlorethylclonidine (CEC) (5×10^{-5} M) was added for 30 minutes. The tissue bath was then washed 5-6 times over a 40 minute interval. A second concentration-response relationship to NE was then obtained. A paired "time control" tissue in each experiment was used to correct for time-dependent changes in agonist sensitivity.

Analysis of Disposition Mechanisms for Norepinephrine

In our experiments we used blockers for neuronal uptake and extraneuronal uptake as well as inhibitors of MAO and COMT to study disposition mechanisms in ring segments of uterine artery The degree of potentiation i.e., the potentiation factor, obtained for norepinephrine produced by blockers and inhibitors of these disposition mechanisms was used as an index of that mechanism in the metabolism of norepinephrine.

Six uterine ring segments from the same uterine artery were prepared and mounted in organ baths containing Krebs solution. The ring segments of uterine artery were equilibrated as described above in section "General Considerations". An initial concentration-response relationship to norepinephrine was obtained by the cumulative addition of norepinephrine in approximately one-half log increments. The tissue baths were then washed 4-5 times at 15 minute intervals, during which time the tissues relaxed and control tensions were achieved. The six ring segments (tissues) were then treated as follows:

(1) Cocaine (10^{-5}M), an uptake-1 blocker, was added to this tissue bath for 20 minutes. This was followed by a second concentration-response relationship to norepinephrine. This will allow us to assess the role that neuronal uptake has in the response to norepinephrine;

(2) Corticosterone (10^{-5}M), an uptake-2 blocker, was added to this tissue bath for a period of 20 minutes. As in tissue 1 above, this was followed by a second concentration-response relationship to norepinephrine. This will allow us to assess the role of extra-neuronal uptake;

(3) Tropolone (10^{-5}M) was added to this tissue bath for a period of 20 minutes. As in the tissues above, this was followed by a second concentration-response relationship to norepinephrine. This will allow us to assess the importance of COMT;

(4) This tissue bath was treated exactly the same way as the three tissues described above, except that no drug was added to it. It was used as a "time control" for the above three tissues in order to correct for any time-dependent changes in the tissue's sensitivity to norepinephrine during the experiment;

(5) To ascertain the involvement of monoamine oxidase, in the disposition of norepinephrine, iproniazid (0.36mM) was added to this tissue bath for 60 minutes. The tissue bath was then washed 5-6 times over a 40 minute period and a second concentration-response relationship to norepinephrine was generated;

(6) This tissue was treated exactly the same way as tissue 5 described above, except that no drug was added to it. It was used as a "time control" for tissue 5.

For each individual, drug i.e., cocaine, corticosterone, tropolone, and iproniazid, the analysis of the data was performed as follows: Effective concentration $(EC_{50})_1$ in the absence of above mentioned drugs was obtained from their concentration-response curve to norepinephrine. Similarly, $(EC_{50})_2$ to norepinephrine in the presence of these drugs was obtained from its concentration-response curve. The shift in the norepinephrine concentration-response curve with time was calculated using the formula (CR_T = EC_{50} at time t/ EC_{50} at time 0) as discussed in the section entitled "Determination of the Dissociation Constant (K_B)". The concentration ratio CR_T

was used to obtain the corrected $(EC_{50})_2$ in the presence of drug using the formula below:

corrected $(EC_{50}) = (EC_{50})_2 / CR_T$.

The potentiation factor (P. F.) was calculated using the formula:

P. F.= corrected $(EC_{50})_2/(EC_{50})_1$.

Statistical Analysis

In order to analyze the data obtained for the determination of the dissociation constant for prazosin, phenylephrine, and WB4101 we used analysis of variance. The data obtained for the subclassification of α_1-adrenergic receptors subtypes and for the disposition study were analyzed by using the Student t-test.

Drugs

The following drugs were used : phenylephrine HCl USP (Winthrop Laboratories); prazosin HCl and nifedipine (Pfizer Inc.); iproniazid phosphate (Hoffman-LaRoche); tropolone (Aldrich Chemical Company); propranolol HCl (Ayrest Laboratories Inc.); cocaine, indomethacin, [-] norepinephrine, and corticosterone 21-acetate (Sigma Chemical Company); dibenamine HCl (Smith, Kline & French); chlorethylclonidine (Research Biochemicals Incorporated); WB4101 (Dr. W. L. Nelson, University of Washington). Medetomidine HCl (Farmos Group Ltd.) All the drugs were dissolved in saline except corticosterone, indomethacin, dibenamine and nifedipine which were dissolved in ethanol.

CHAPTER 4. RESULTS

Phenylephrine produced concentration-dependent contractions of porcine uterine artery during the luteal phase, follicular phase and in early pregnancy stages. Prazosin, an α_1-adrenergic receptor antagonist, competitively inhibited responses to phenylephrine in the luteal phase, follicular phase as well as in early pregnancy. Maximum responses to phenylephrine were not affected by prazosin (3×10^{-9}, 10^{-8}, 3×10^{-8} M) (Figures 4.1, 4.2 and 4.3). Schild plots (Figures 4.4, 4.5, and 4.6) for prazosin *vs* phenylephrine yield straight line during the luteal phase, follicular phase and early pregnancy with slopes not significantly different from unity. The pA_2 values obtained in the luteal phase, in the follicular phase and in the early pregnancy were 8.98, 9.04, and 9.10 respectively. These pA_2 values are not significantly different from each other ($P > 0.05$). The dissociation constant (K_B) was found to be 10.4×10^{-10}M, 9.1×10^{-10}M, and 7.9×10^{-10}M in the luteal phase, in the follicular phase and in early pregnancy stages, respectively.

In order to ascertain the contribution that α_2-adrenergic receptors may have in mediating contractile responses to norepinephrine, the α_2-adrenergic receptor agonist medetomidine was used. Medetomidine did not produce any significant contraction. The threshhold response for contraction to medetomidine was observed at the concentration of 3×10^{-7}-10^{-6}M and the maximum response to medetomidine

was observed at a concentration of 10^{-5}M. The maximum response obtained (to 10^{-5}M) of medetomidine was 18% relative to the initial response to phenylephrine. (N= 4,results not shown).

An example of the experiment to determine the K_A for phenylephrine is presented in Figure 4.7. Dibenamine reduced the maximal response to phenylephrine about 40%-60% in the luteal phase, in the follicular phase the response was reduced by 25%-75% and in the early pregnancy maximal response was reduced by 50%-75%. The mean dissociation constant (K_A) for phenylephrine was found to be 6.5 $\pm$ 1.8 x 10^{-6}M in luteal phase, 3.7 $\pm$ 0.7 x 10^{-6}M in follicular phase and 4.4 $\pm$ 1.7 x 10^{-6}M in early pregnancy. These K_A values are not significantly different from each other(P > 0.05).

Norepinephrine produced concentration-dependent contractions in isolated uterine artery during the luteal phase, follicular phase, and in early pregnancy stages. The putative selective α_{1A}-adrenergic antagonist WB4101 (10^{-8}M) inhibited responses to norepinephrine in the luteal phase, follicular phase and in early pregnancy (Figures 4.8, 4.9, and 4.10). The dissociation constant (K_B) in the luteal phase, follicular phase and in early pregnancy was found to be 11.7 x 10^{-10}M, 1.69 x 10^{-10}M. 5.4 x 10^{-10}M, respectively. These K_B values are not significantly different from each other (P > 0.05).

The selective irreversible α_{1B} -adrenergic antagonist CEC (10^{-5}M) displaced the norepinephrine concentration-response relationship curve to the right and also significantly depressed the maximum response to norepinephrine in the luteal phase, follicular phase and in early pregnancy (Figures 4.11, 4.12, and 4.13). The percent maximal reduction to norepinephrine in the presence of CEC was 44% in the luteal

phase, 30.5% in the follicular phase, and 41% in early pregnancy.

The calcium channel antagonist nifedipine (10^{-6}M) shifted the concentration-response curve to norepinephrine to the right in the luteal phase, follicular phase, and in early pregnancy. However, the shift was significant only in the follicular phase. The maximum contractile response to norepinephrine was reduced by 26.1% in the luteal phase, 41.5% in the follicular phase, and 16.4% in early pregnancy by nifedipine (Figures 4.14, 4.15, and 4.16).

Table 4.1: The potentiation of responses to NE by drugs which influence its disposition

Pregnancy stage	Drug	Potentiating Factor
luteal phase	Iproniazid	$1.15 \pm .17^{a}$
	Cocaine	$.38 \pm .05^{b}$
	Corticosterone	$.23 \pm .04^{b}$
	Tropolone	$1.0 \pm .21^{a}$
Follicular phase	Iproniazid	$.76 \pm .07^{a}$
	Cocaine	$.37 \pm .05^{b}$
	Corticosterone	$.48 \pm .14^{b}$
	Tropolone	$.99 \pm .23^{a}$
Early pregnancy	Iproniazid	$.94 \pm .36^{a}$
	Cocaine	$.47 \pm .10^{b}$
	Corticosterone	$.41 \pm .08^{b}$
	Tropolone	$1.1 \pm .23^{a}$

[a]Superscript not different from 1 ($P > 0.05$)
[b]Superscript different from 1 ($P < 0.01$)

The potentiation factor obtained for iproniazid, (Figures 4.17, 4.18, and 4.19), was not significantly different from unity in the luteal phase, follicular phase and in early pregnant pigs ($P > 0.05$). Results are summarized in (Table 4.1). Similar results were obtained with tropolone where the potentiation factor was not significantly

different from unity in the luteal phase, follicular phase and in early pregnancy ($P > 0.05$). Results are summarized in (Table 4.1). However, the potentiation factor obtained for cocaine was significantly different from unity in the luteal phase, follicular phase and in early pregnancy ($P < 0.01$) The potentiation factor obtained for corticosterone in the luteal phase, follicular phase and in early pregnancy was significantly different by unity (Figures 4.20, 4.21, and 4.22) ($P < 0.01$). Ethanol(20 μ1) did not have any effect on norepinephrine- induced contractions. ($P > 0.05$, Figure 4.23).

Figure 4.1. Cumulative concentration-response curves for phenylephrine (PE) obtained on isolated rings of porcine uterine artery in the luteal phase before and after equilibration for 60 minutes in the presence of prazosin (3×10^{-9}, 1×10^{-8}, and 3×10^{-8}M). Each point represents the mean $\pm$ S.E of tissues from 6 animals and is expressed as a percentage of the control contraction obtained to phenylephrine (3×10^{-6}M).

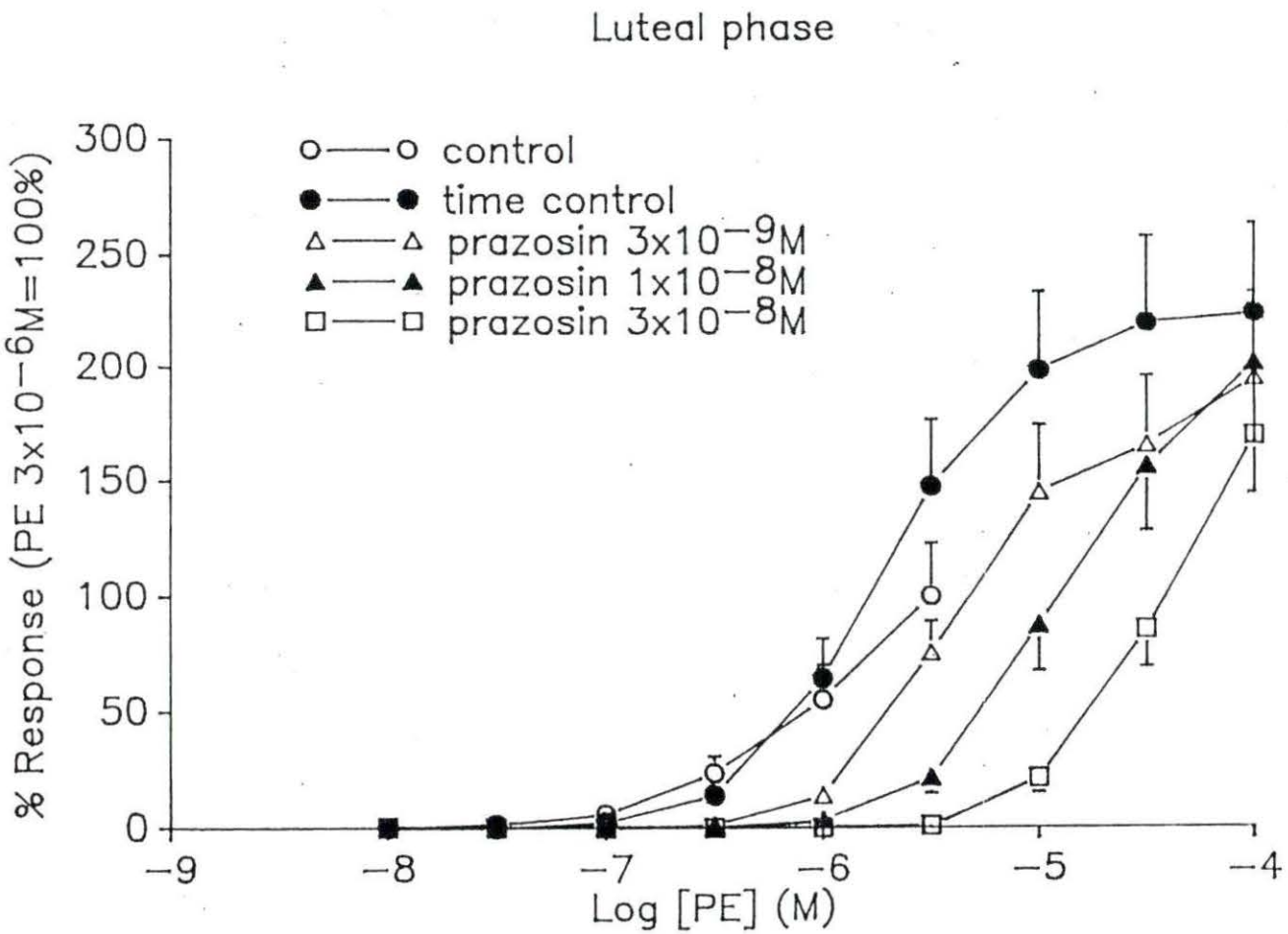

Luteal phase
control
time control
prazosin 3x10⁻⁹M
prazosin 1x10⁻⁸M
prazosin 3x10⁻⁸M
% Response (PE 3x10⁻⁶M=100%)
300
250
200
150
100
50
0
−9
−8
−7
−6
−5
−4
Log [PE] (M)

Figure 4.2. Cumulative concentration-response curves for phenylephrine (PE) obtained on isolated rings of porcine uterine artery in the follicular phase before and after equilibration for 60 minutes in the presence of prazosin (3×10^{-9}, 1×10^{-8}, and 3×10^{-8}M). Each point represents the mean $\pm$ S.E of tissues from 6 animals and is expressed as a percentage of the control contraction obtained to phenylephrine (3×10^{-6}M).

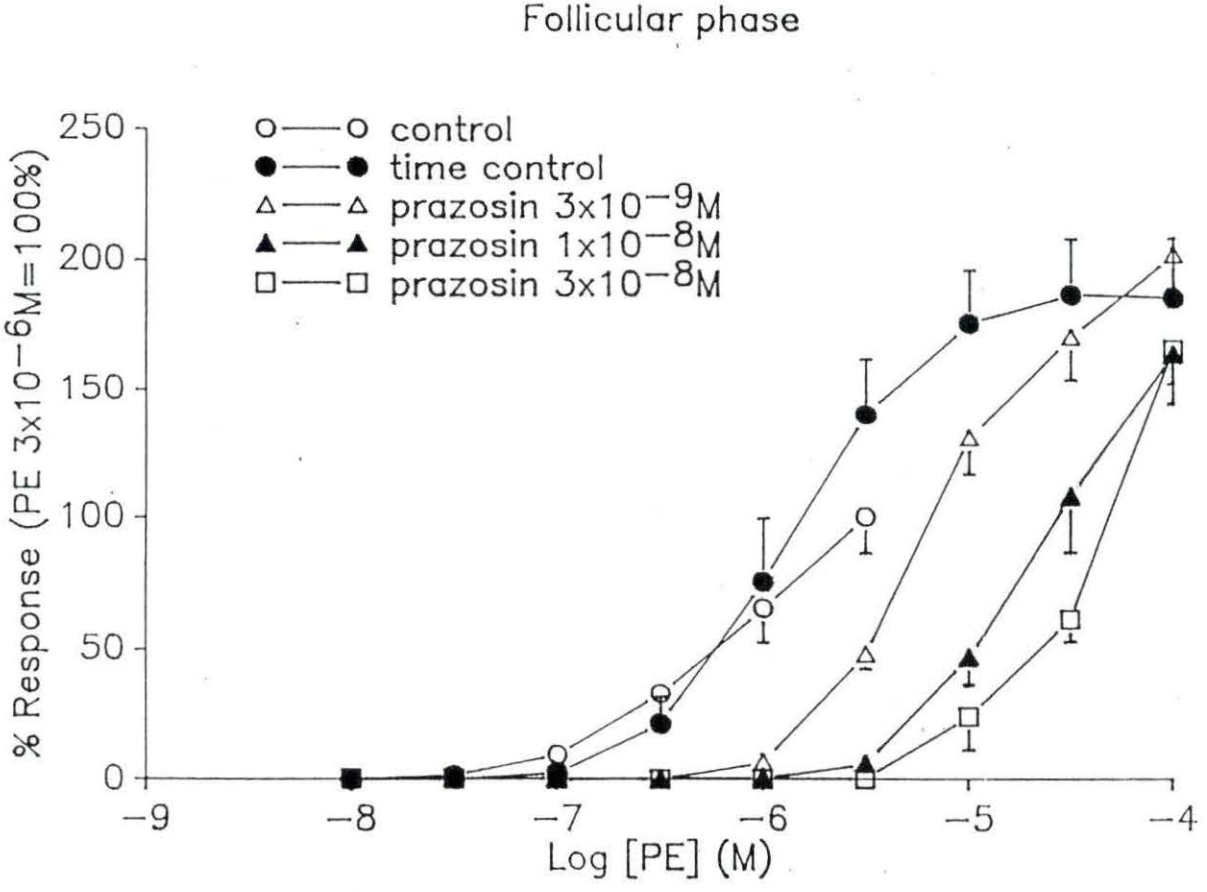

Follicular phase
control
time control
prazosin 3x10⁻⁹M
prazosin 1x10⁻⁸M
prazosin 3x10⁻⁸M
% Response (PE 3x10⁻⁶M=100%)
250
200
150
100
50
0
−9
−8
−7
−6
−5
−4
Log [PE] (M)

Figure 4.3. Cumulative concentration-response curves for phenylephrine (PE) obtained on isolated rings of porcine uterine artery in the early pregnancy before and after equilibration for 60 minutes in the presence of prazosin (3×10^{-9}, 1×10^{-8}, and 3×10^{-8}M). Each point represents the mean $\pm$ S.E of tissues from 6 animals and is expressed as a percentage of the control contraction obtained to phenylephrine (3×10^{-6}M).

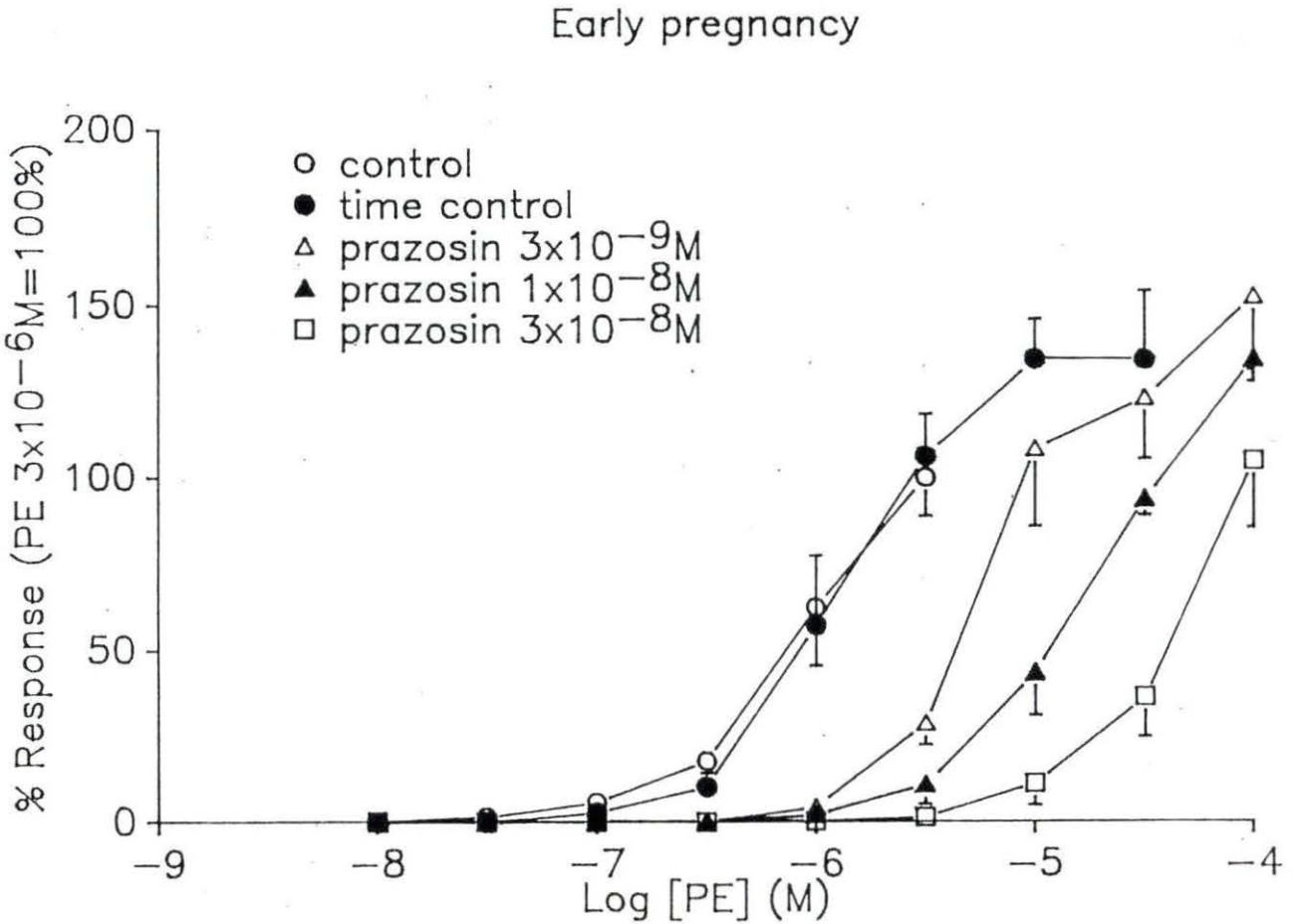

Early pregnancy
control
time control
prazosin 3x10−9M
prazosin 1x10−8M
prazosin 3x10−8M
% Response (PE 3x10−6M=100%)
200
150
100
50
0
−9
−8
−7
−6
−5
−4
Log [PE] (M)

Figure 4.4. Schild plot for determination of the pA_2 for prazosin when tested against phenylephrine on isolated rings of porcine uterine artery in the luteal phase. Each point represents the mean of six experiments from 6 animals. The intercept on the abscissa gives the pA_2 value. The slope of the fitted regression line is shown in the figure.

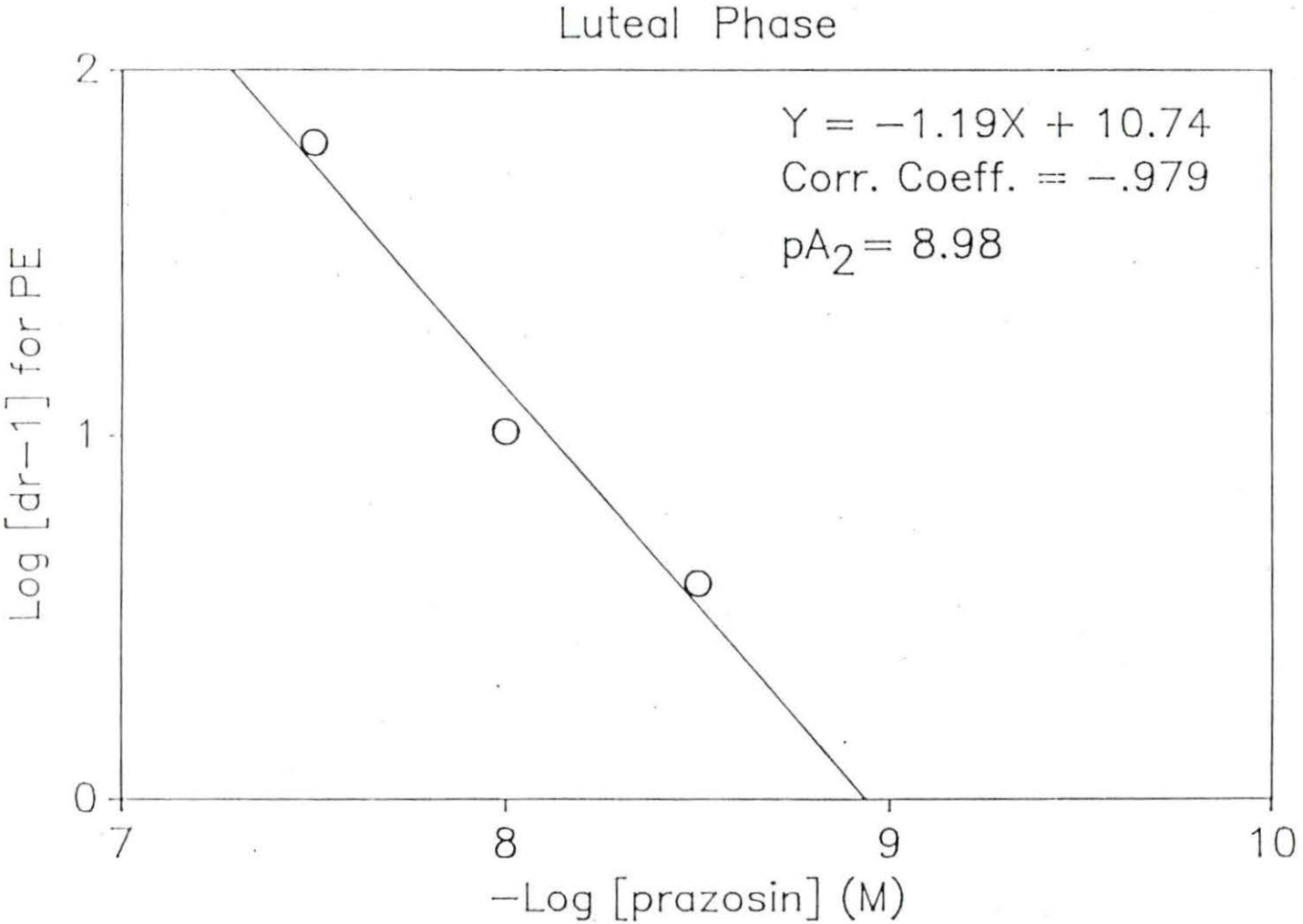

Luteal Phase
Y = −1.19X + 10.74
Corr. Coeff. = −.979
pA2 = 8.98
Log [dr−1] for PE
2
1
0
7
8
9
10
−Log [prazosin] (M)

Figure 4.5. Schild plot for determination of the pA_2 for prazosin when tested against phenylephrine on isolated rings of porcine uterine artery 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.

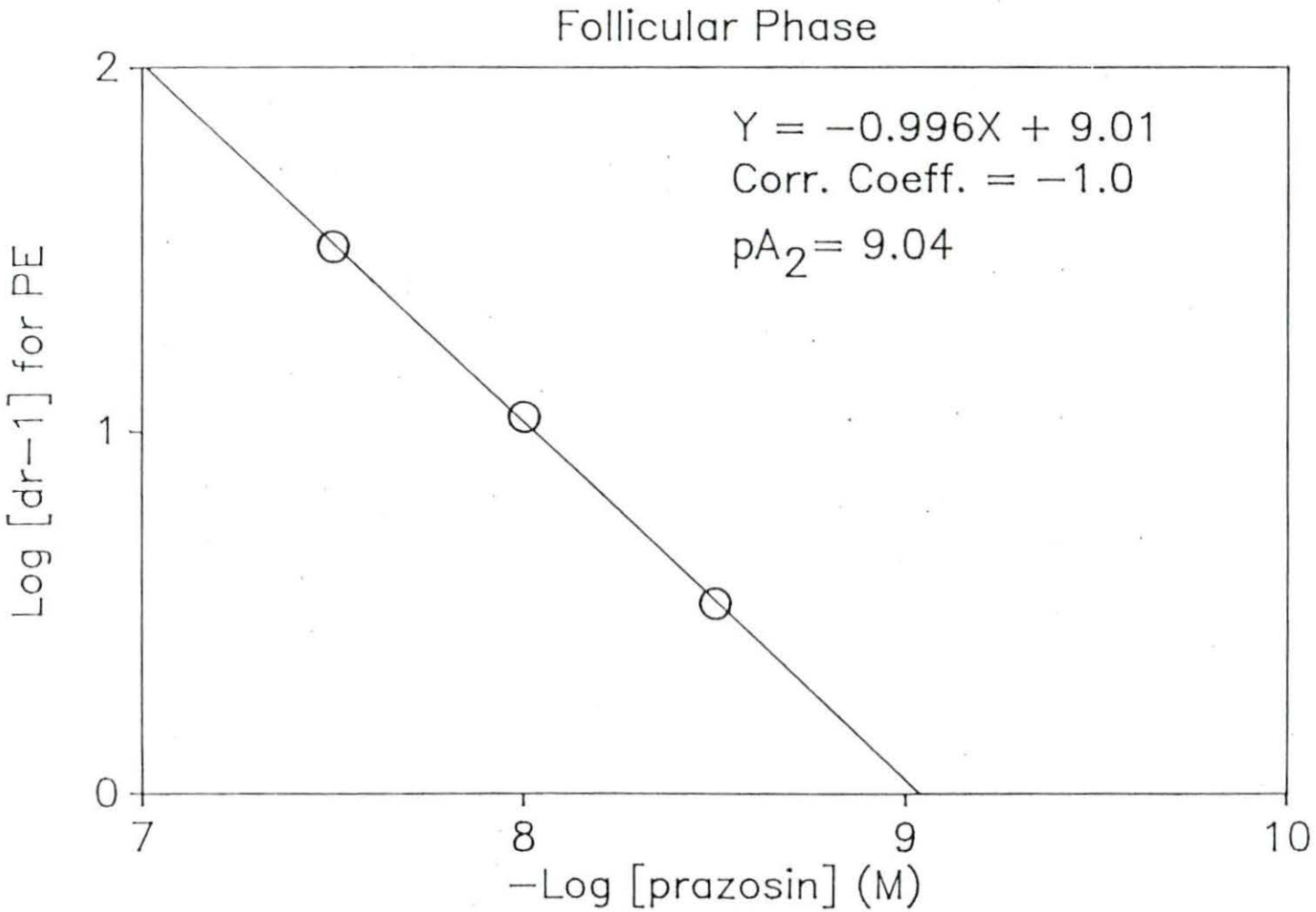

Follicular Phase
Y = −0.996X + 9.01
Corr. Coeff. = −1.0
pA2= 9.04
Log [dr−1] for PE
2
1
0
7
8
9
10
−Log [prazosin] (M)

Figure 4.6. Schild plot for determination of the pA_2 for prazosin when tested against phenylephrine on isolated rings of porcine uterine artery in early pregnancy. Each point represents the average of the six experiments. The intercept on the abscissa gives the pA_2 value. The slope of the fitted regression line is shown in the figure.

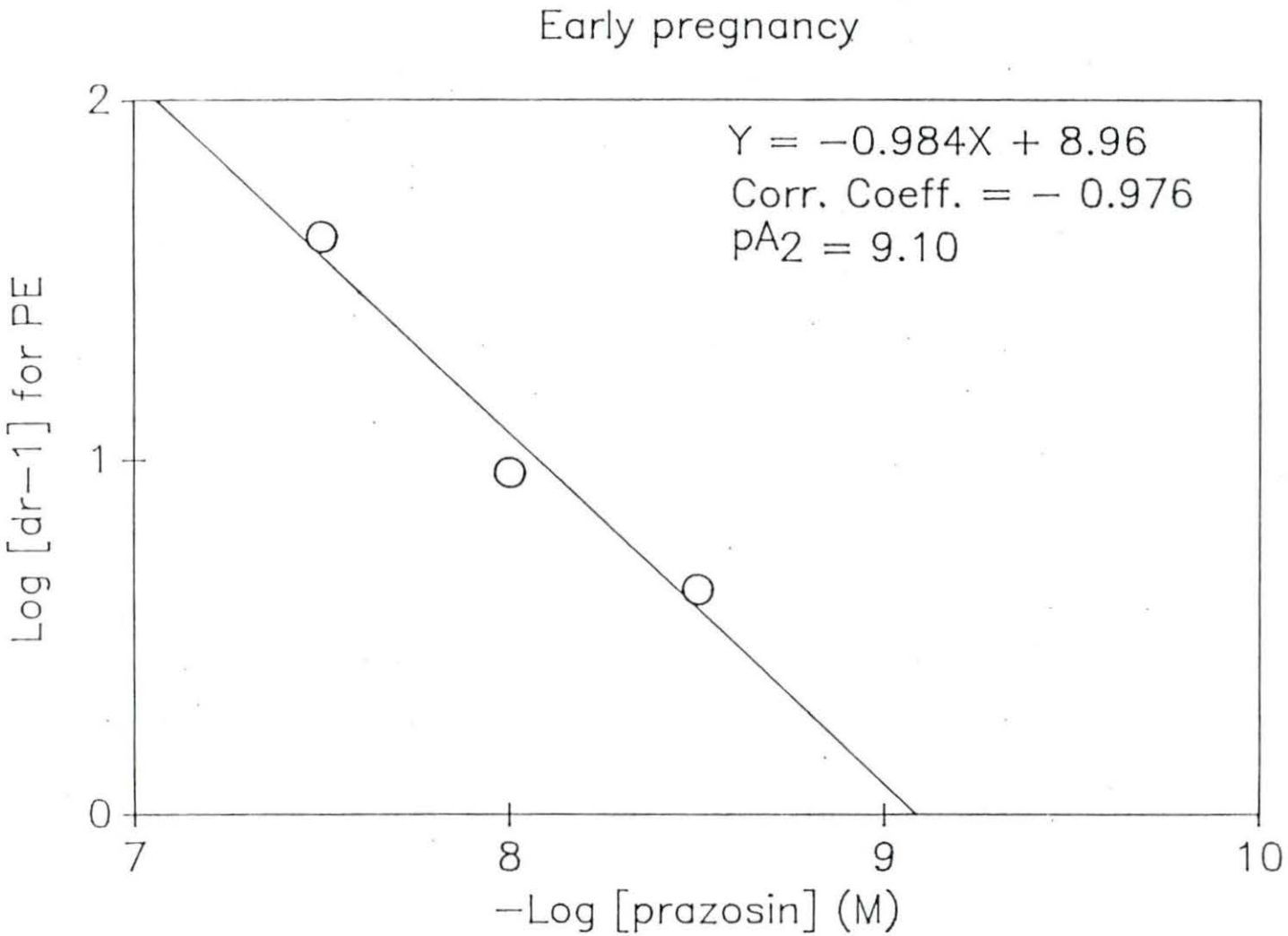
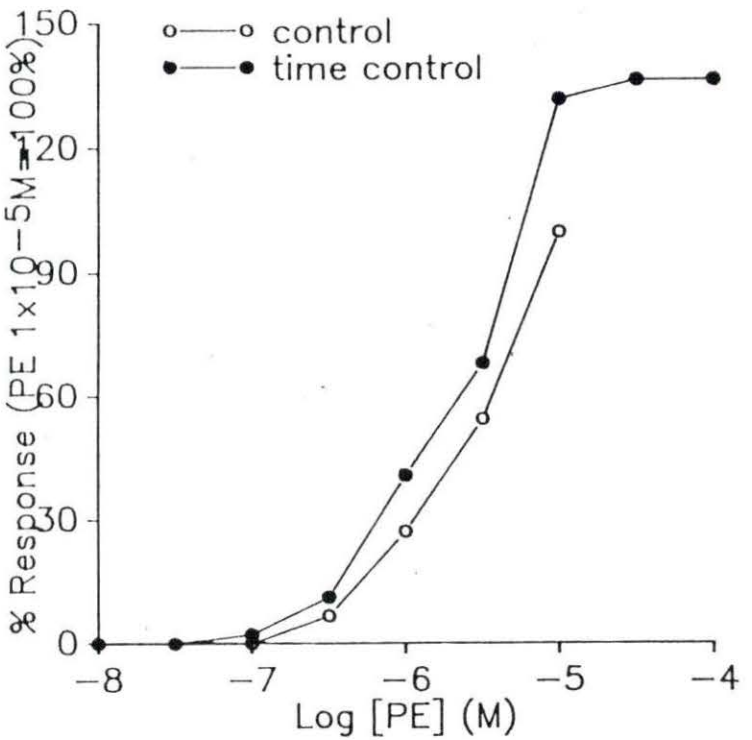

Early pregnancy
Y = −0.984X + 8.96
Corr. Coeff. = − 0.976
pA2 = 9.10
Log [dr−1] for PE
2
1
0
7
8
9
10
−Log [prazosin] (M)

Figure 4.7. An example of the effect of treatment with dibenamine on phenylephrine elicited contractions on isolated rings of porcine uterine artery in the follicular phase. Contractions to phenylephrine were obtained before and after exposure to (7.5×10^{-7}M of dibenamine) for 20 minutes. Dibenamine was washed out of the tissues before obtaining the second concentration-relationship to phenylephrine.

Follicular phase



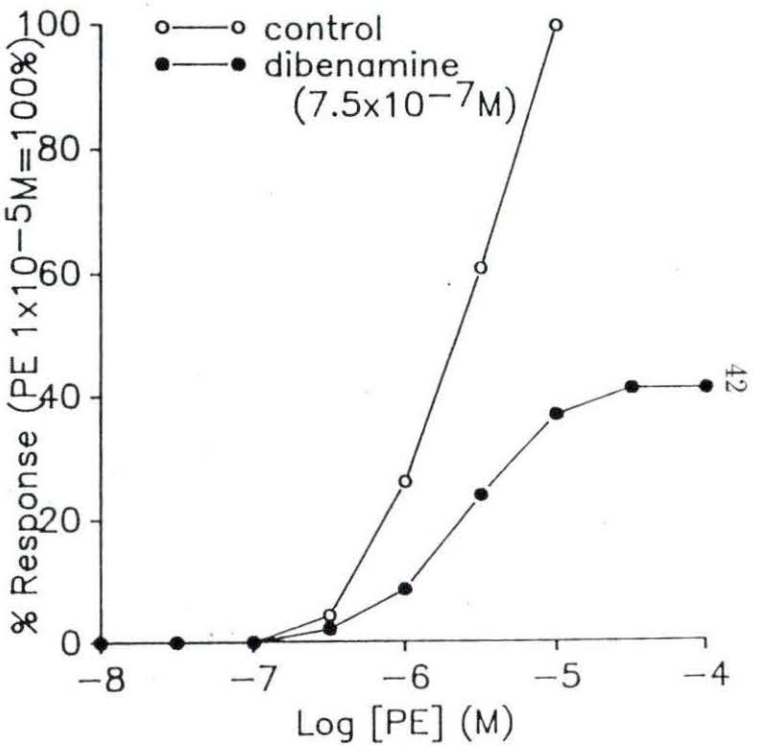


Figure 4.8. Effect of WB4101 10^{-8}M on norepinephrine-induced contractions of isolated porcine uterine artery in the luteal phase. Each point represents the mean $\pm$ S.E of experiments from five animals. Points with asterisk (*) are significantly different from time control ($P < 0.05$).

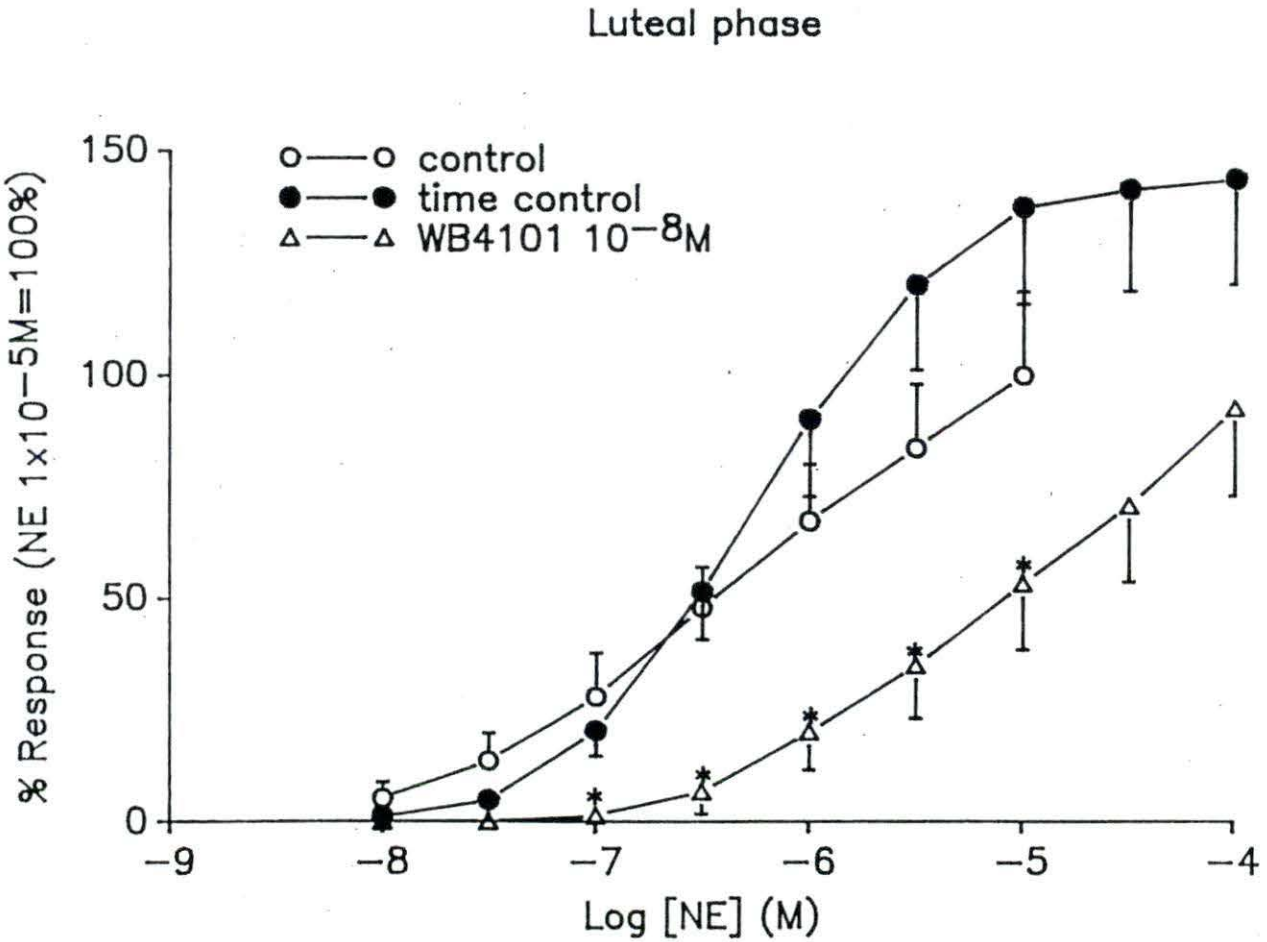

Luteal phase
control
time control
WB4101 10−8M
% Response (NE 1x10−5M=100%)
150
100
50
0
−9
−8
−7
−6
−5
−4
Log [NE] (M)

Figure 4.9. Effect of WB4101 10^{-8}M on norepinephrine-induced contractions of isolated porcine uterine artery in the follicular phase. Each point represents the mean $\pm$ S.E of experiments from five animals. Points with asterisk (*) are significantly different from time control ($P < 0.05$).

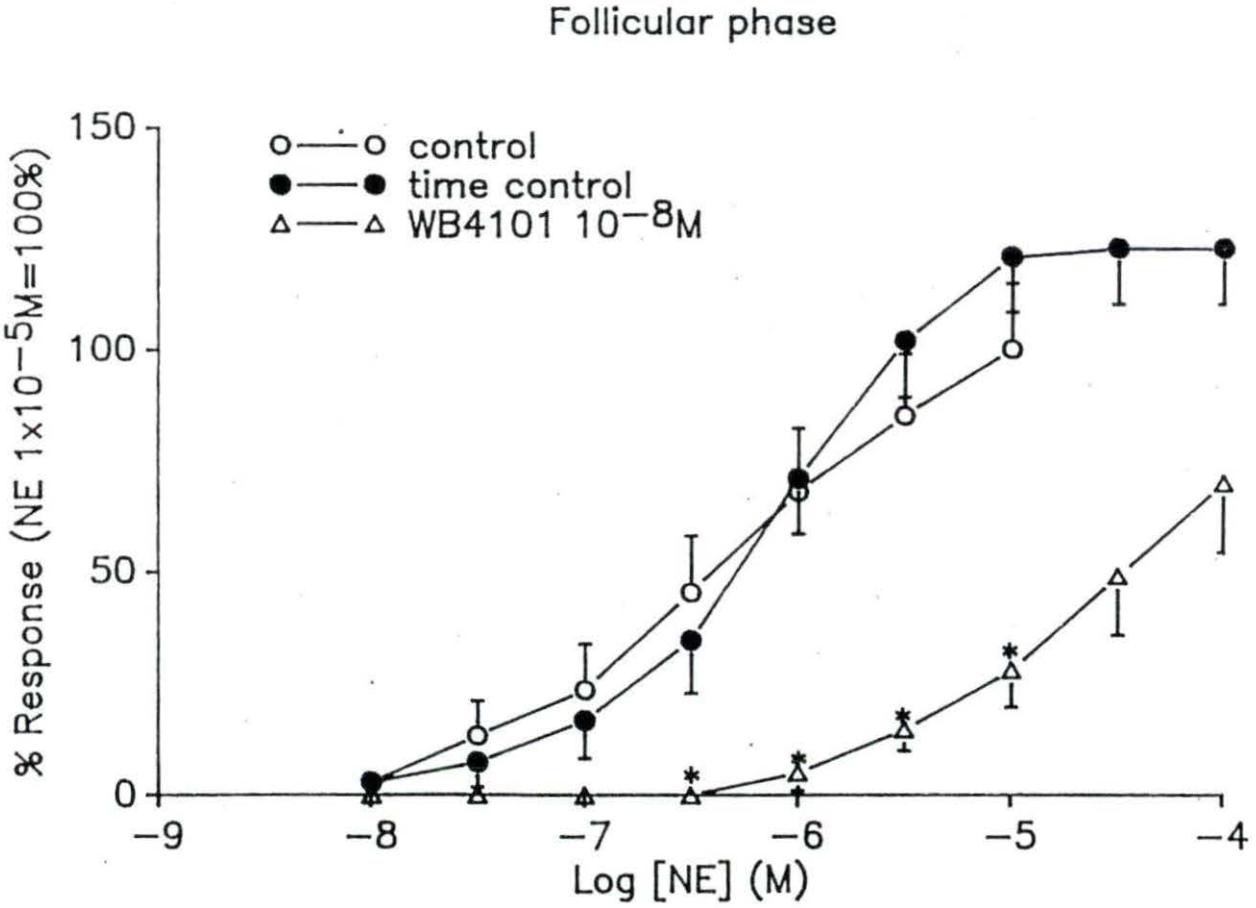

Follicular phase
control
time control
WB4101 10−8M
% Response (NE 1x10−5M=100%)
150
100
50
0
−9
−8
−7
−6
−5
−4
Log [NE] (M)

Figure 4.10. Effect of WB4101 10^{-8}M on norepinephrine-induced contractions on isolated porcine uterine artery in early pregnancy. Each point represents the mean $\pm$ S.E of experiments from five animals. Points with asterisk (*) are significantly different from time control ($P < 0.05$).

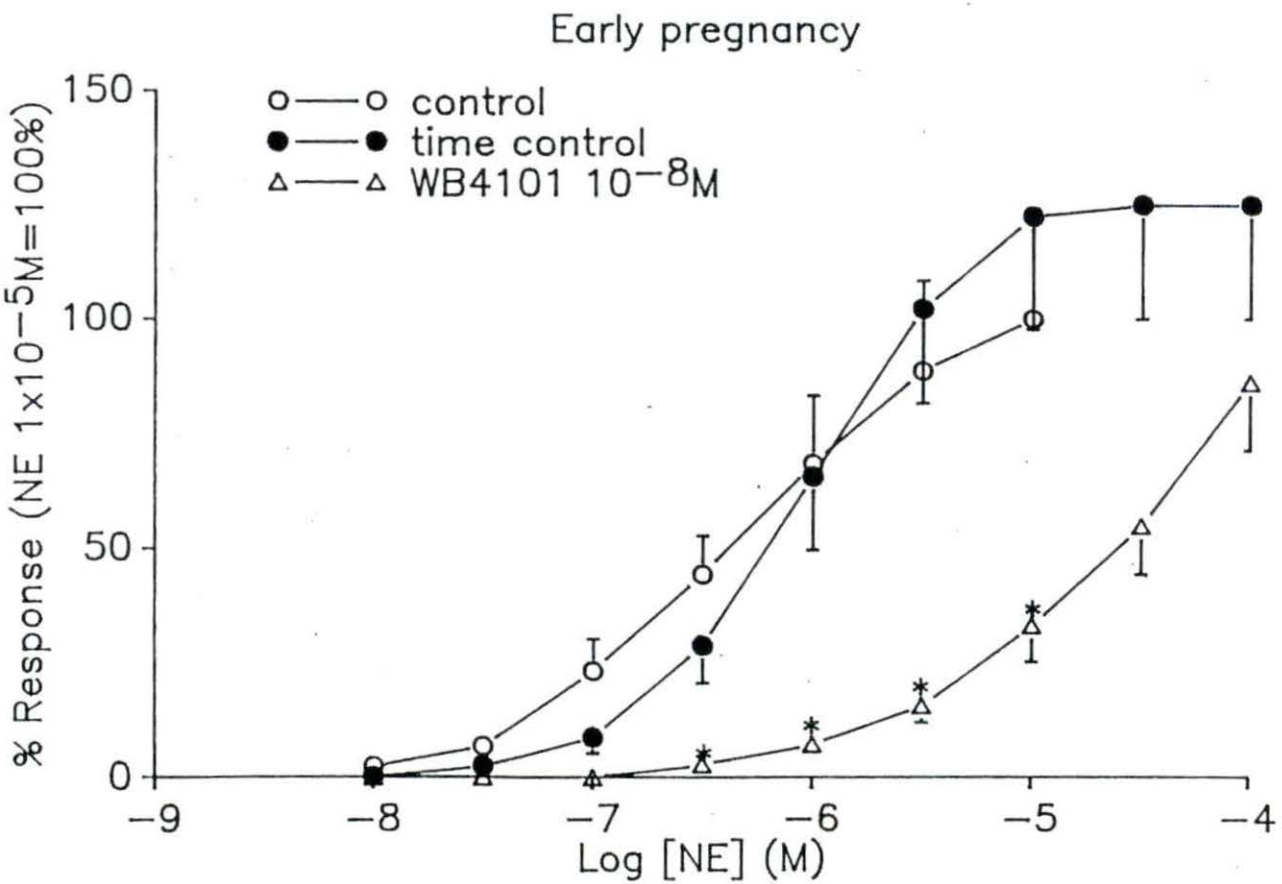

Early pregnancy
control
time control
WB4101 10−8M
% Response (NE 1x10−5M=100%)
150
100
50
0
−9
−8
−7
−6
−5
−4
Log [NE] (M)

Figure 4.11. Effect of chlorethylclonidine 5 x 10^{-5}M on norepinephrine -induced contractions of isolated porcine uterine artery in the luteal phase. Each point represents the mean $\pm$ S.E of experiments from five animals. Points with asterisk (*) are significantly different from time control ($P < 0.05$).

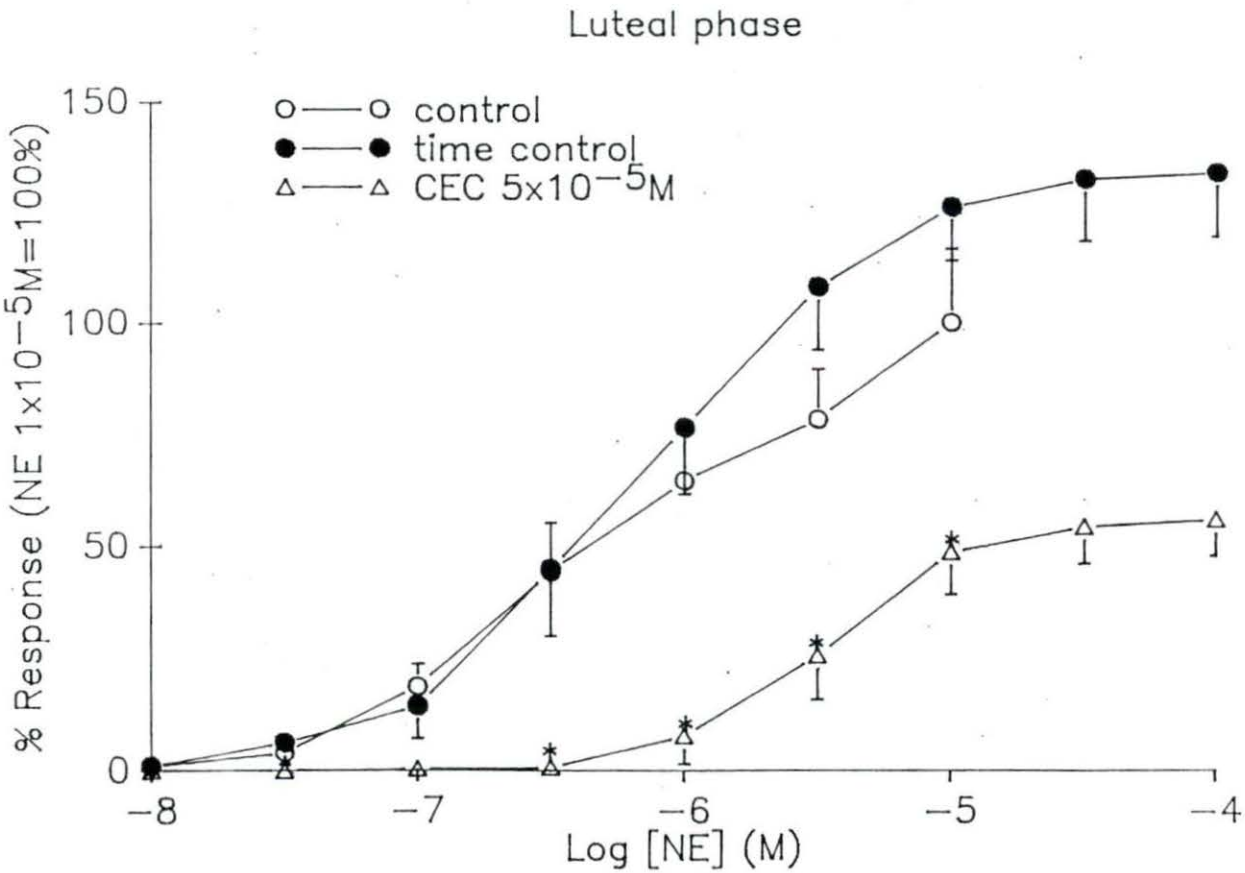

Luteal phase
control
time control
CEC 5x10⁻⁵M
% Response (NE 1x10⁻⁵M=100%)
150
100
50
0
−8
−7
−6
−5
−4
Log [NE] (M)

Figure 4.12. Effect of chlorethylclonidine 5 x 10^{-5}M on norepinephrine -induced contractions of isolated porcine uterine artery in the follicular phase. Each point represents the mean $\pm$ S.E of experiments from five animals. Points with asterisk (*) are significantly different from time control ($P < 0.05$).

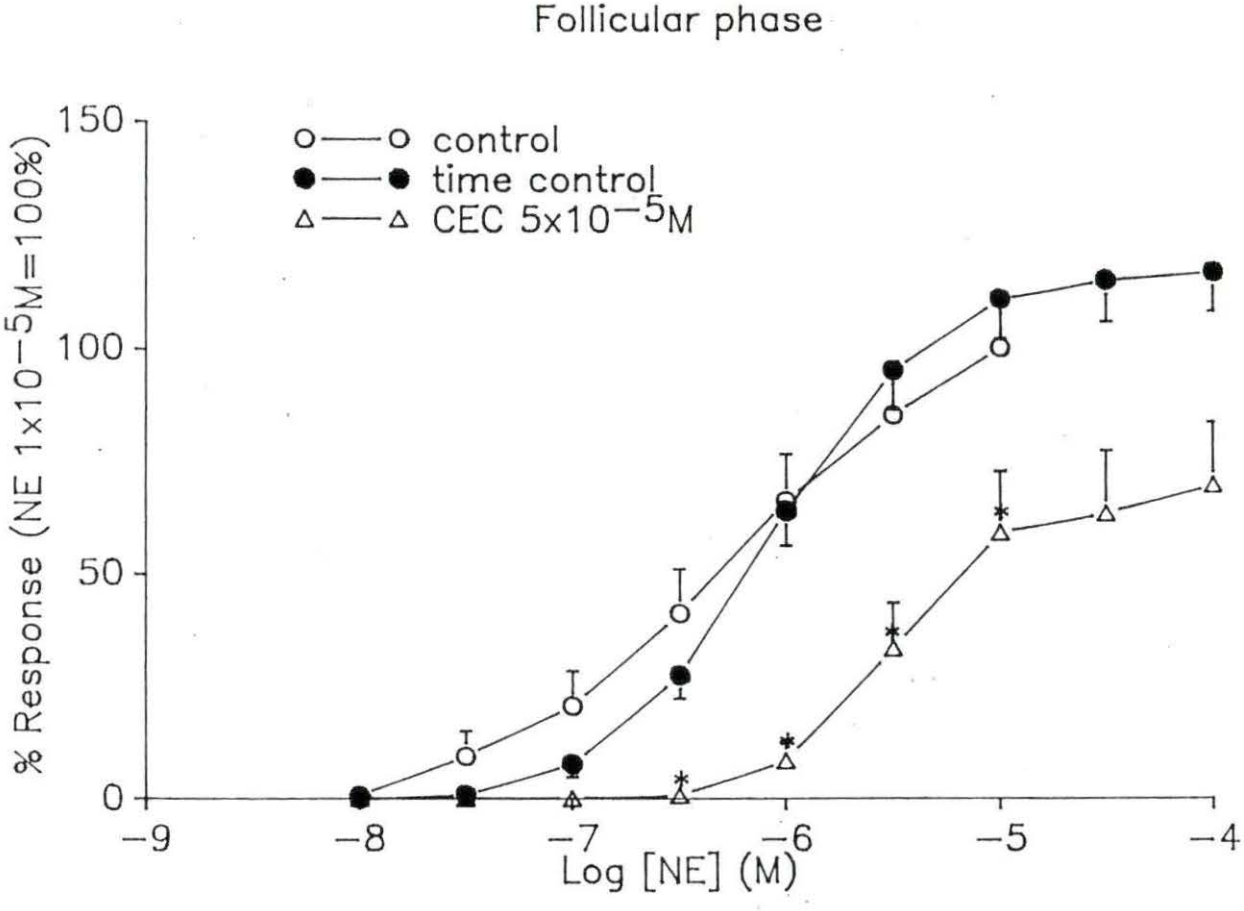

Follicular phase
control
time control
CEC 5x10⁻⁵M
% Response (NE 1x10⁻⁵M=100%)
150
100
50
0
−9
−8
−7
−6
−5
−4
Log [NE] (M)

Figure 4.13. Effect of chlorethylclonidine 5 x 10^{-5}M on norepinephrine-induced contractions of isolated porcine uterine artery in early pregnancy. Each point represents the mean ± S.E of experiments from five animals. Points with asterisk (*) are significantly different from time control ($P < 0.05$).

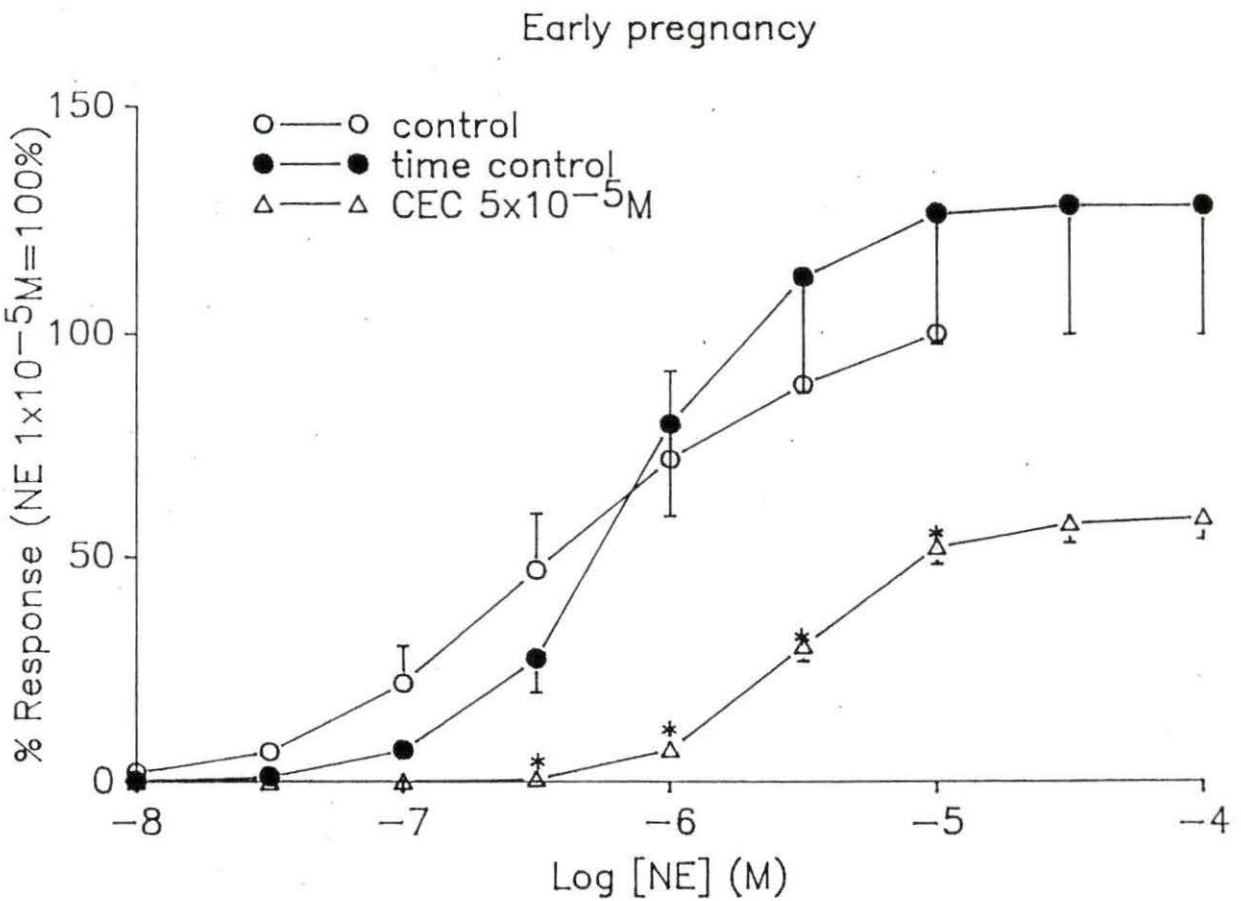

Early pregnancy
control
time control
CEC 5x10⁻⁵M
% Response (NE 1x10⁻⁵M=100%)
150
100
50
0
−8
−7
−6
−5
−4
Log [NE] (M)

Figure 4.14. Effect of nifedipine 10^{-6}M on norepinephrine -induced contractions of isolated porcine uterine artery in the luteal phase. Each point represents the mean ± S.E of experiments from four animals. None of the points on the nifedipine curve are significantly different from the time control.

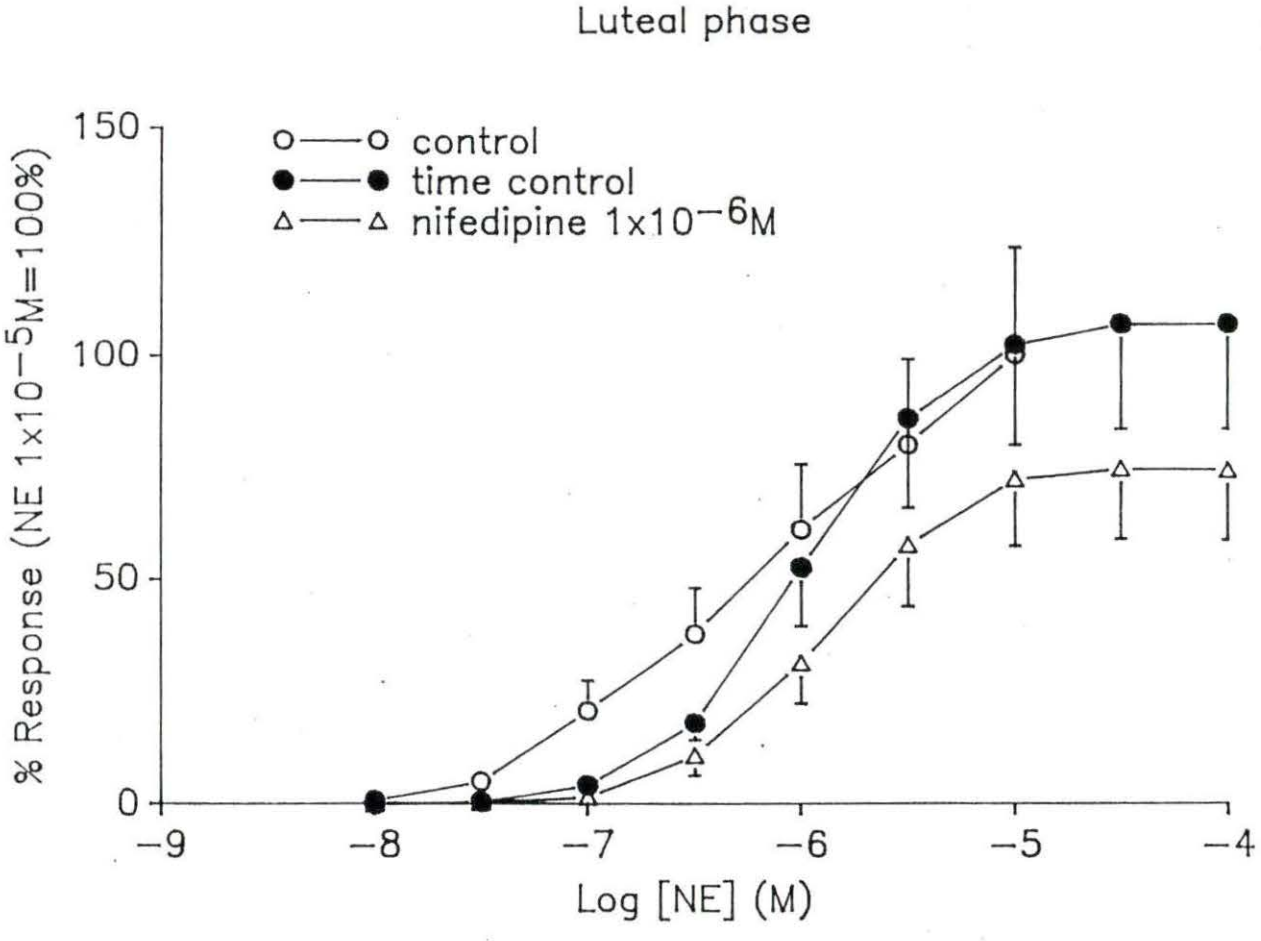

Luteal phase
control
time control
nifedipine 1x10⁻⁶M
% Response (NE 1x10⁻⁵M=100%)
150
100
50
0
−9
−8
−7
−6
−5
−4
Log [NE] (M)

Figure 4.15. Effect of nifedipine 10^{-6}M on norepinephrine-induced contractions of isolated porcine uterine artery in the follicular phase. Each point represents the mean $\pm$ S.E of experiments from four animals. Points with asterisk (*) are significantly different from time control ($P < 0.05$).

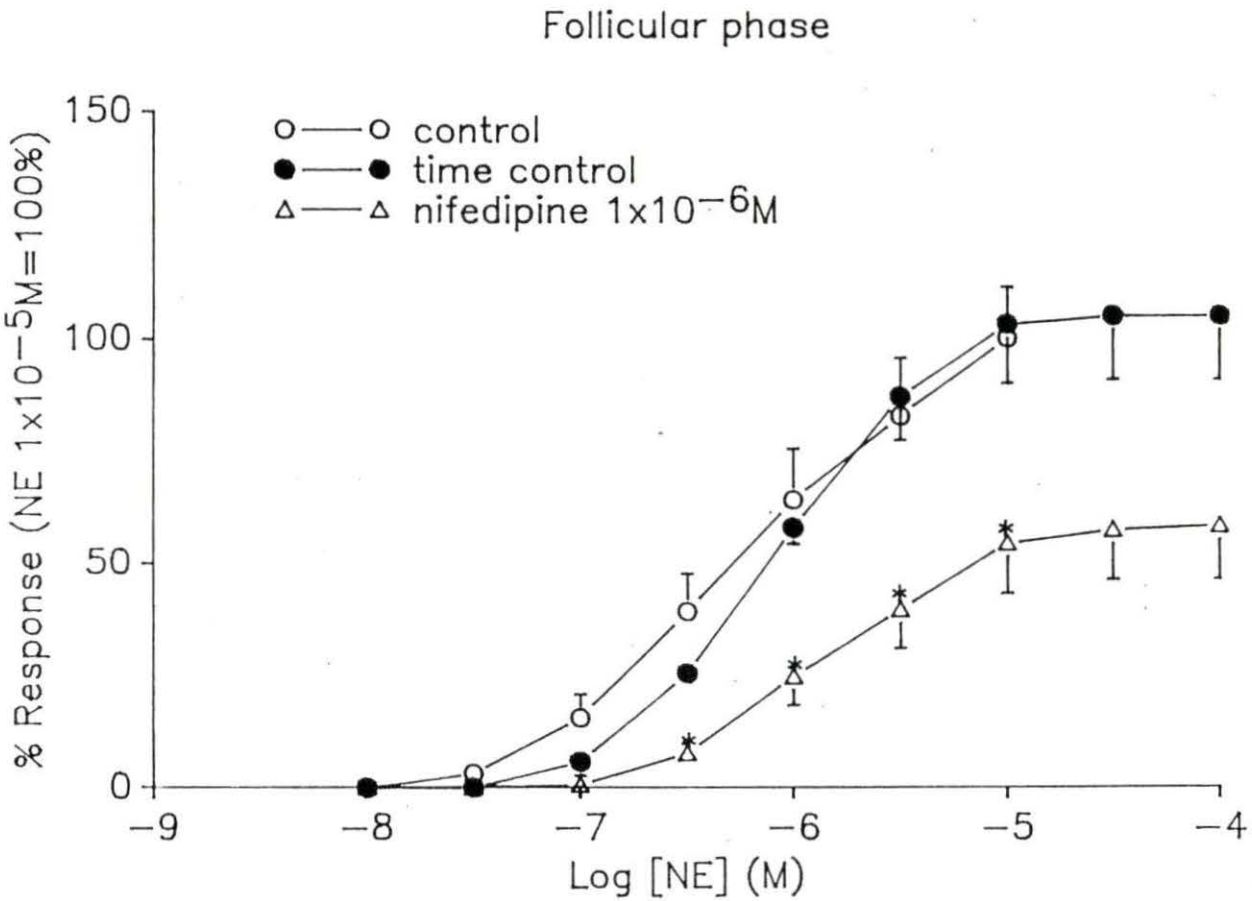

Follicular phase
o—o control
●—● time control
△—△ nifedipine 1x10⁻⁶M
% Response (NE 1x10⁻⁵M=100%)
150
100
50
0
−9
−8
−7
−6
−5
−4
Log [NE] (M)

Figure 4.16. Effect of nifedipine 10^{-6}M on norepinephrine-induced contractions of isolated porcine uterine artery in the early pregnancy. Each point represents the mean $\pm$ S.E of experiments from four animals. None of the points on the nifedipine curve are significantly different from the time control.

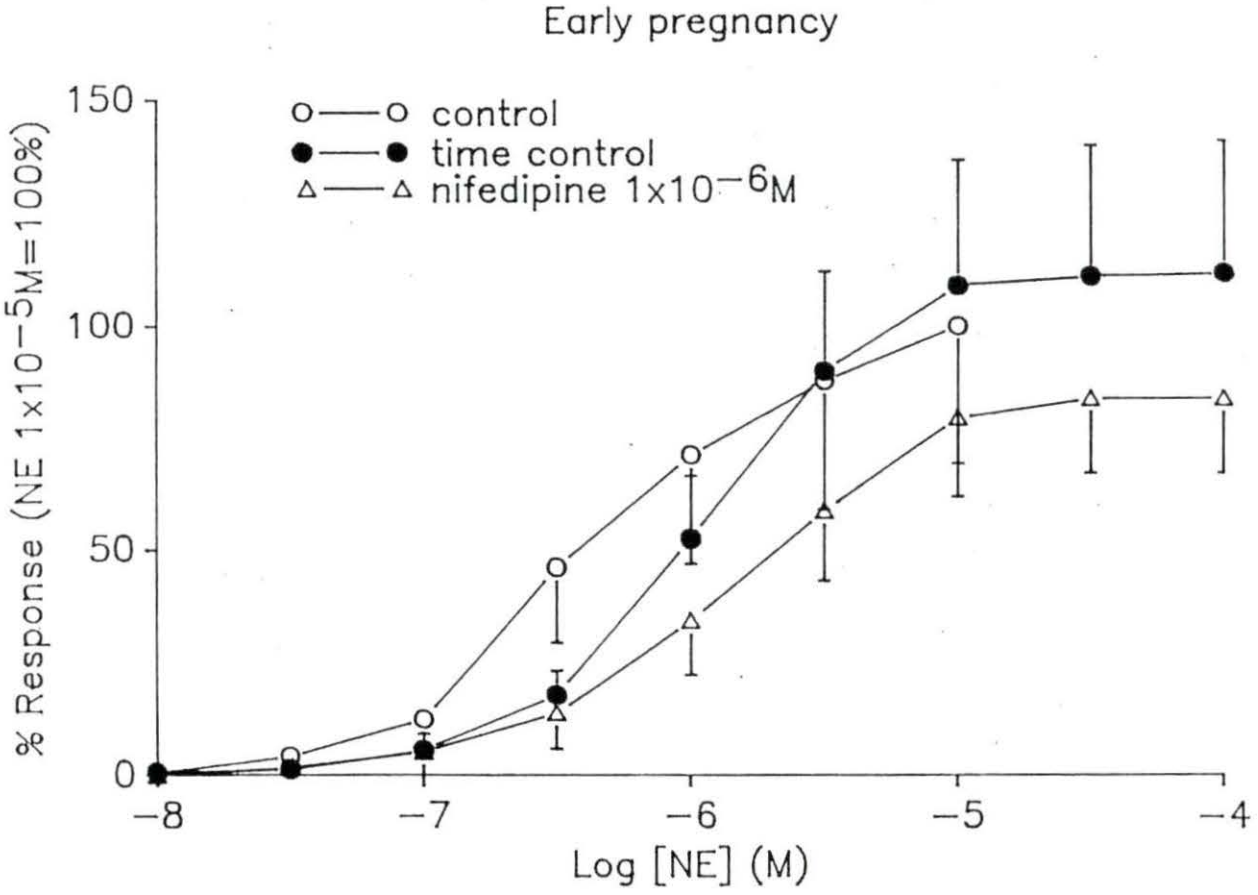

Early pregnancy
o—o control
●—● time control
△—△ nifedipine 1x10⁻⁶M
% Response (NE 1x10⁻⁵M=100%)
150
100
50
0
−8
−7
−6
−5
−4
Log [NE] (M)

Figure 4.17. Effect of iproniazid 0.36mM on norepinephrine-induced contractions of isolated porcine uterine artery in the luteal phase. Iproniazid was added for 60 minutes as described in methods. Each bar represents the mean ± S.E of experiments from five animals. These data were used to determine the EC_{50} which was then used to calculate the potentiation factor.

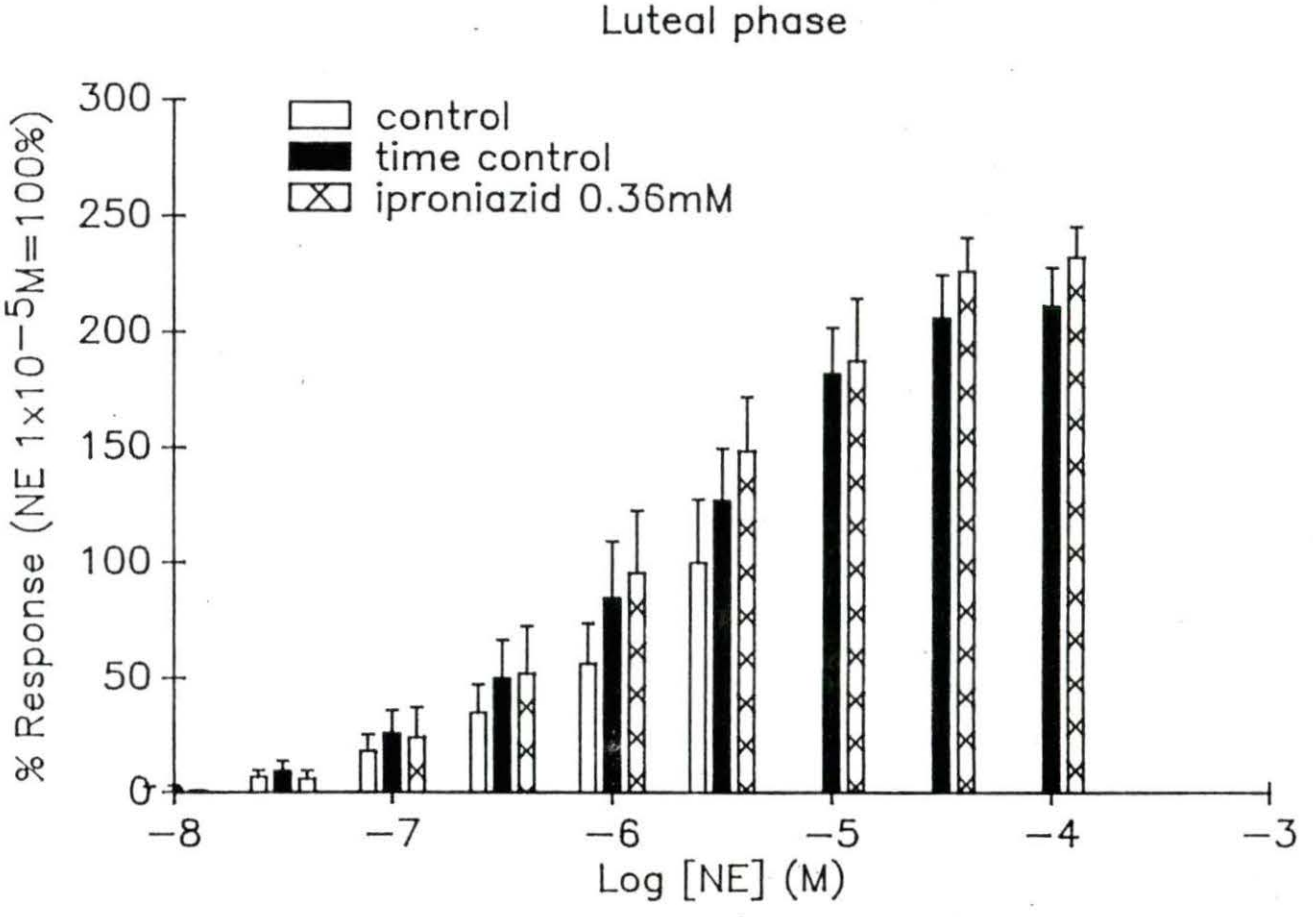

Luteal phase
control
time control
iproniazid 0.36mM
% Response (NE 1x10−5M=100%)
300
250
200
150
100
50
0
−8
−7
−6
−5
−4
−3
Log [NE] (M)

Figure 4.18. Effect of iproniazid 0.36mM on norepinephrine-induced contractions of isolated porcine uterine artery in the follicular phase. Iproniazid was added for 60 minutes as described in methods. Each bar represents the mean ± S.E of experiments from five animals. These data were used to determine the EC_{50} which was then used to calculate the potentiation factor.

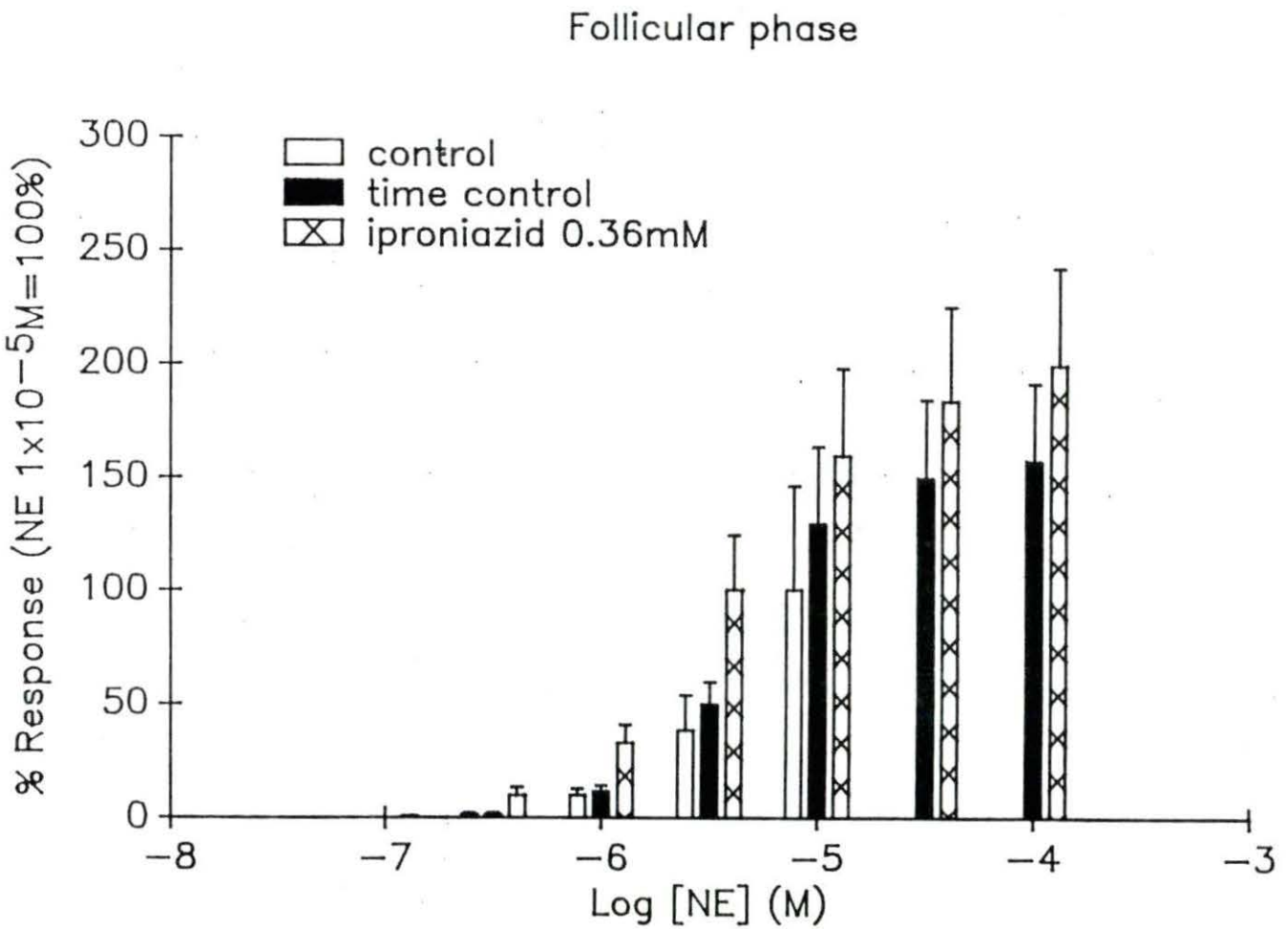

Follicular phase
control
time control
iproniazid 0.36mM
% Response (NE 1x10−5M=100%)
300
250
200
150
100
50
0
−8
−7
−6
−5
−4
−3
Log [NE] (M)

Figure 4.19. Effect of iproniazid 0.36mM on norepinephrine-induced contractions of isolated porcine uterine artery in the early pregnancy. Iproniazid was added for 60 minutes as described in methods. Each bar represents the mean $\pm$ S.E of experiments from five animals. These data were used to determine the EC_{50} which was then used to calculate the potentiation factor.

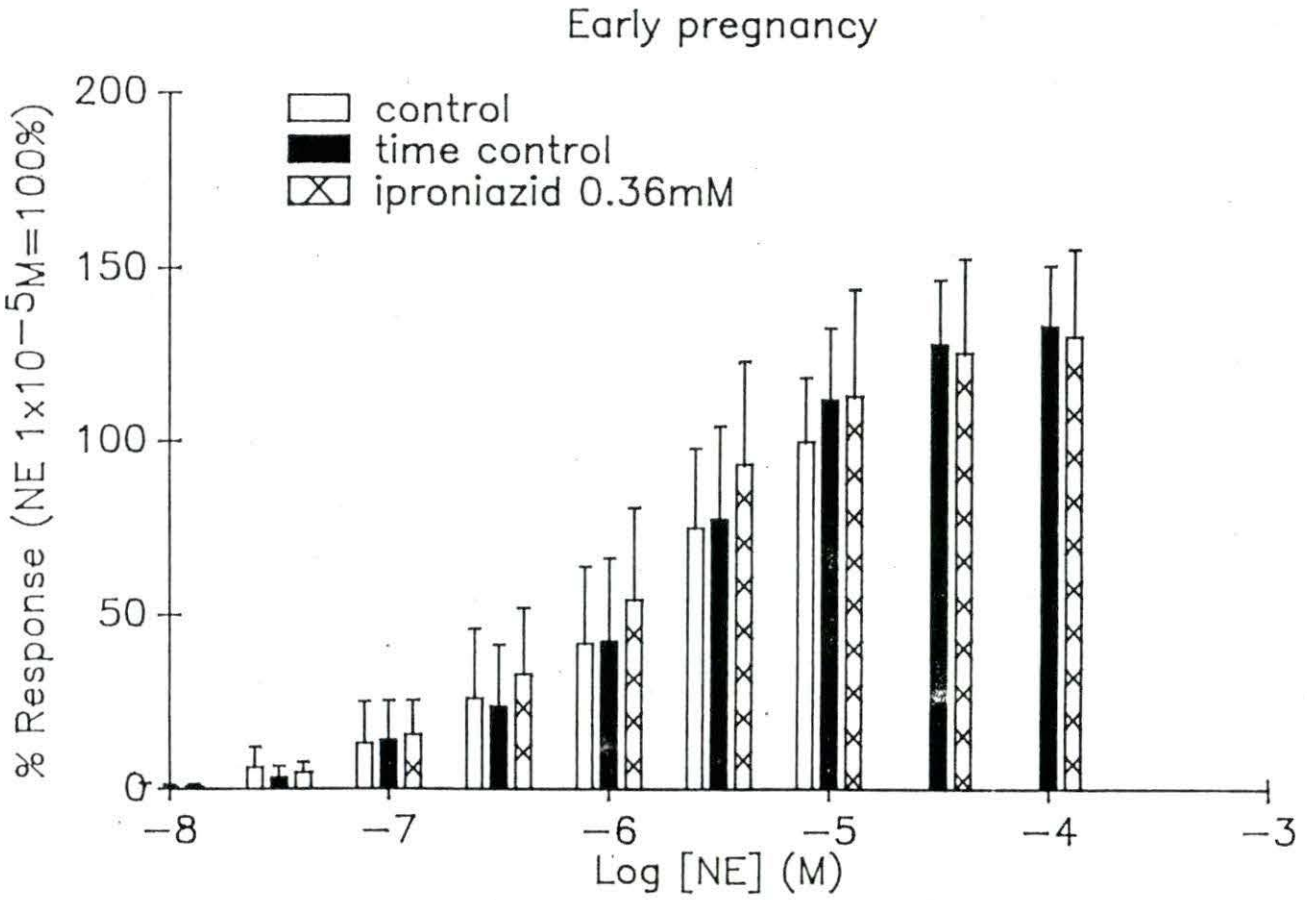

Early pregnancy
control
time control
iproniazid 0.36mM
% Response (NE 1x10−5M=100%)
200
150
100
50
0
−8
−7
−6
−5
−4
−3
Log [NE] (M)

Figure 4.20. Effect of cocaine 10^{-5}M, corticosterone 10^{-5}M, and tropolone 10^{-5}M on norepinephrine-induced contractions of isolated porcine uterine artery in the luteal phase. Cocaine, corticosterone, and tropolone were added for 20 minutes as described in methods. Each bar represents the mean $\pm$ S.E of experiments from five animals. These data were used to determine the EC_{50} which was then used to calculate the potentiation factor.

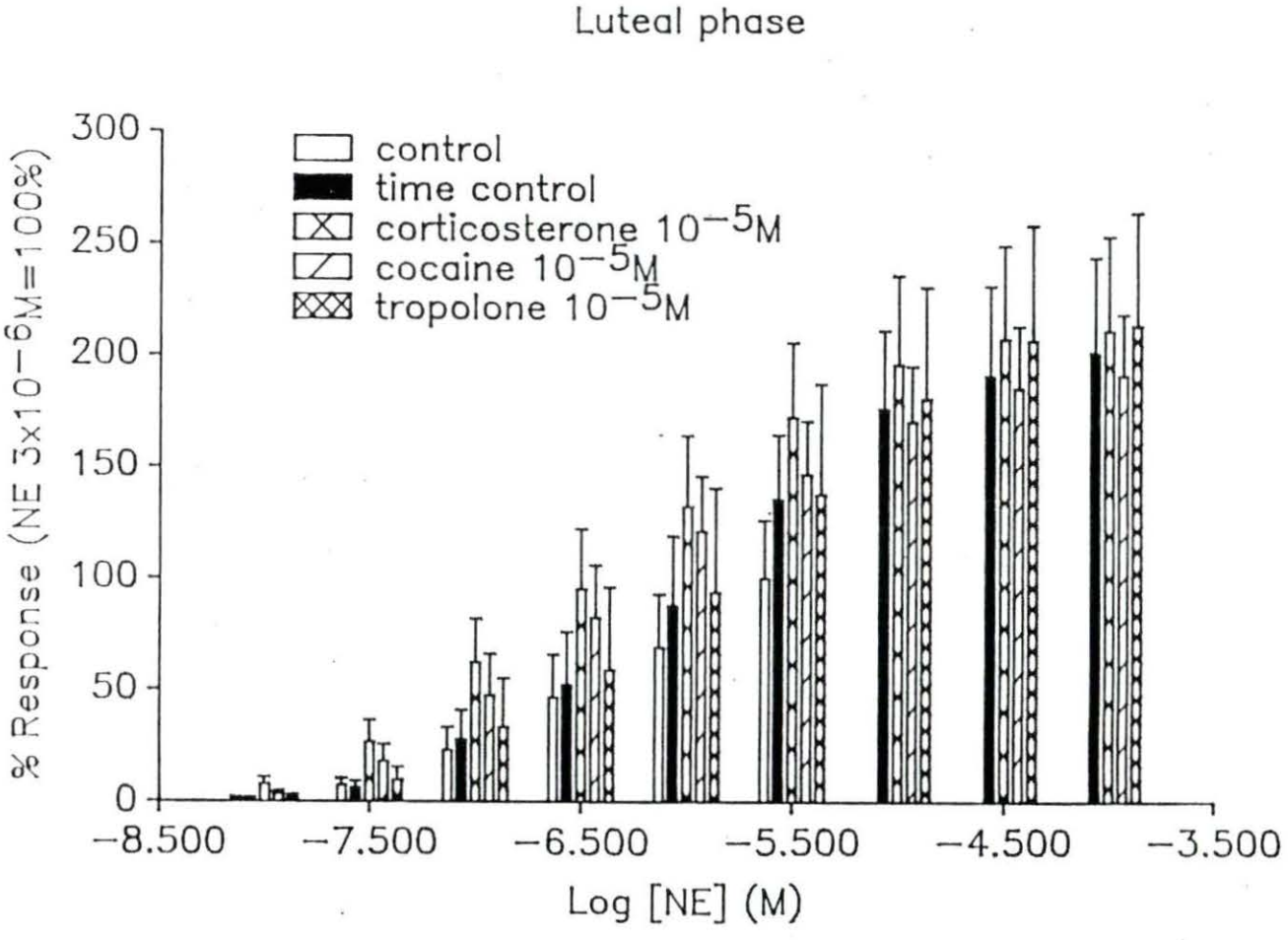

Luteal phase
control
time control
corticosterone 10−5M
cocaine 10−5M
tropolone 10−5M
% Response (NE 3x10−6M=100%)
300
250
200
150
100
50
0
−8.500
−7.500
−6.500
−5.500
−4.500
−3.500
Log [NE] (M)

Figure 4.21. Effect of cocaine 10^{-5}M, corticosterone 10^{-5}M, and tropolone 10^{-5}M on norepinephrine-induced contractions of isolated porcine uterine artery in the follicular phase. Cocaine, corticosterone, and tropolone were added for 20 minutes as described in methods. Each bar represents the mean $\pm$ S.E of experiments from five animals. These data were used to determine the EC_{50} which was then used to calculate the potentiation factor.

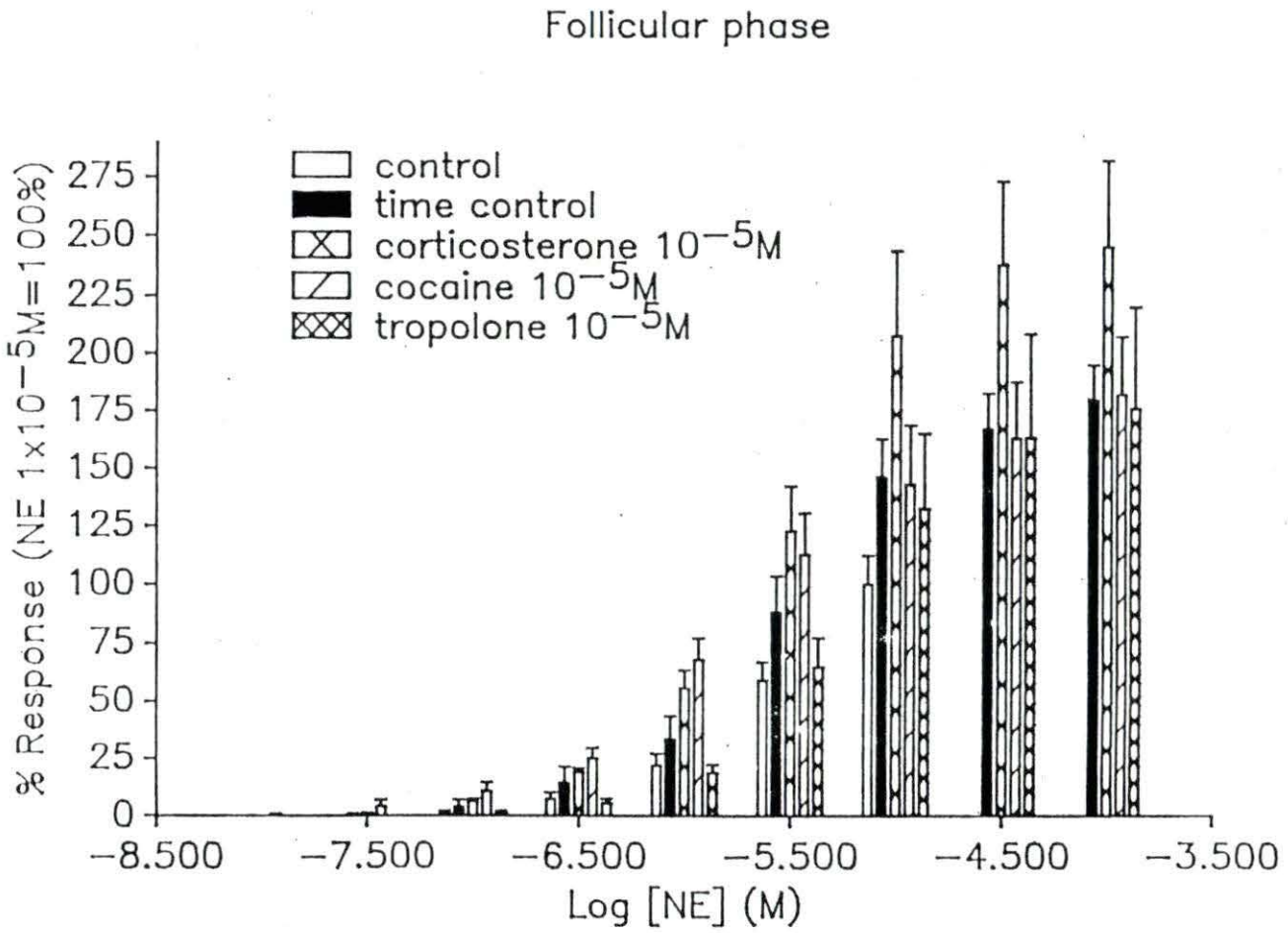

Follicular phase
control
time control
corticosterone 10⁻⁵M
cocaine 10⁻⁵M
tropolone 10⁻⁵M
% Response (NE 1x10⁻⁵M=100%)
275
250
225
200
175
150
125
100
75
50
25
0
−8.500
−7.500
−6.500
−5.500
−4.500
−3.500
Log [NE] (M)

Figure 4.22. Effect of cocaine 10^{-5}M, corticosterone 10^{-5}M, and tropolone 10^{-5}M on norepinephrine-induced contractions of isolated porcine uterine artery in early pregnancy. Cocaine, corticosterone, and tropolone were added for 20 minutes as described in methods. Each bar represents the mean $\pm$ S.E of experiments from five animals. These data were used to determine the EC_{50} which was then used to calculate the potentiation factor.

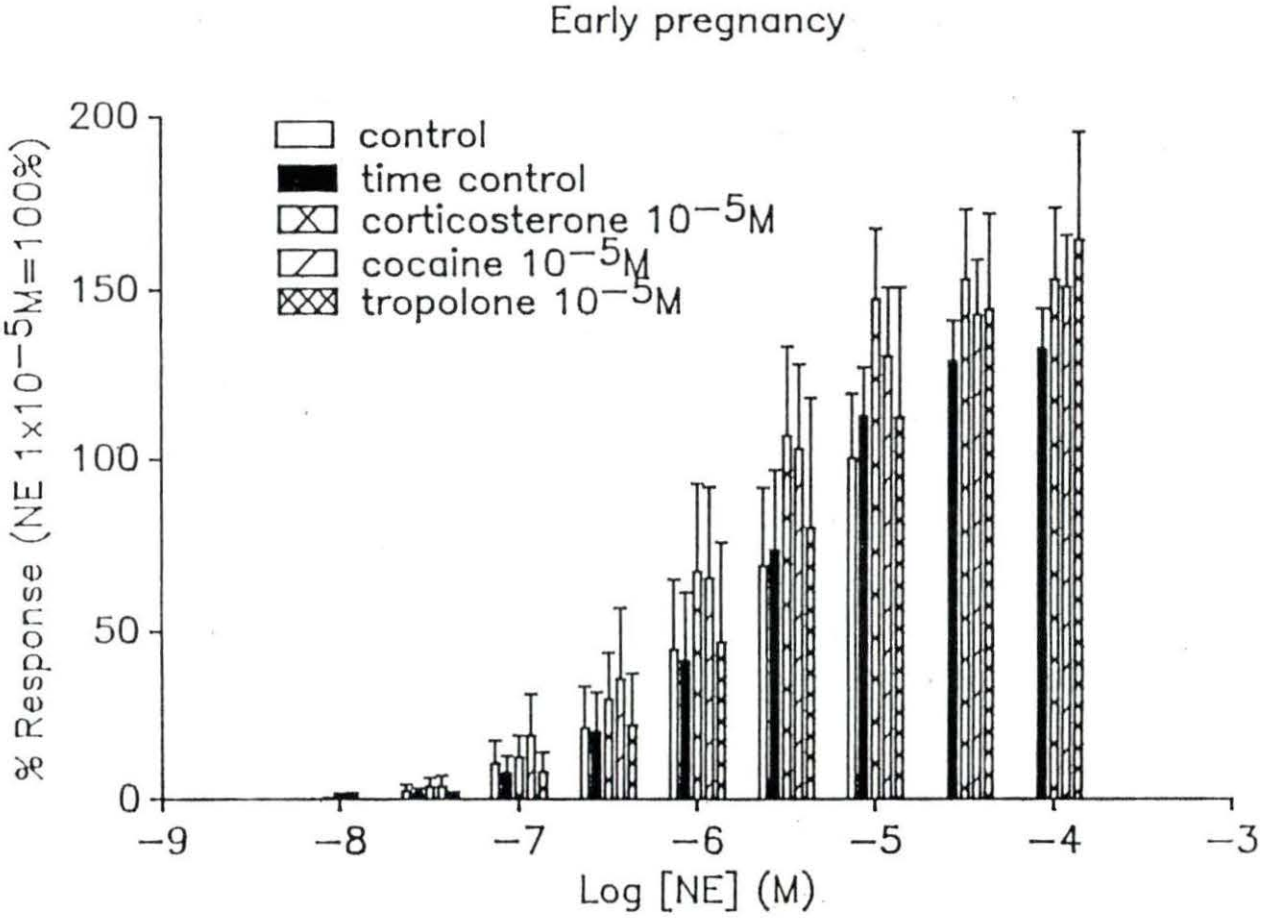
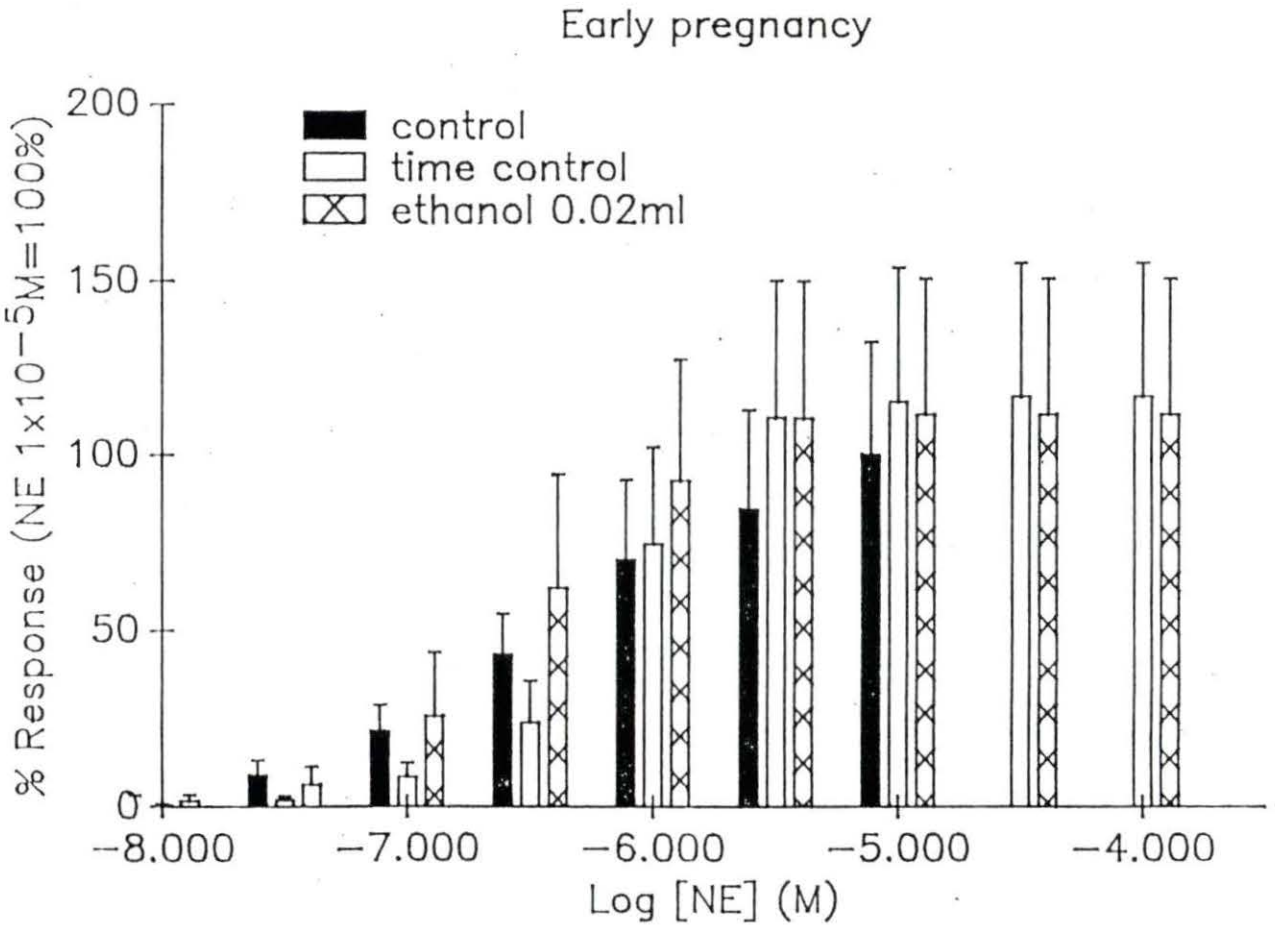

Early pregnancy
control
time control
corticosterone 10−5M
cocaine 10−5M
tropolone 10−5M
% Response (NE 1x10−5M=100%)
200
150
100
50
0
−9
−8
−7
−6
−5
−4
−3
Log [NE] (M)

Figure 4.23. Effect of ethanol (20 μl) on norepinephrine-induced contractions of isolated porcine uterine artery in early pregnancy. Each bar represents the mean $\pm$ S.E of four experiments.


Early pregnancy
control
time control
ethanol 0.02ml
% Response (NE 1x10−5M=100%)
200
150
100
50
0
−8.000
−7.000
−6.000
−5.000
−4.000
Log [NE] (M)

CHAPTER 5. DISCUSSION

Determination of the Dissociation Constant for Prazosin

In our study we have classified α_1-adrenergic receptors in isolated porcine uterine artery in the luteal phase, follicular phase and in early pregnancy, on the basis of their sensitivity to phenylephrine, a selective α_1-adrenergic receptor agonist, and prazosin, a selective α_1-adrenergic receptor antagonist. Selective antagonists are widely used to classify receptors because a single property, affinity, governs their interaction with the receptor [44]. Prazosin is generally accepted as a potent and highly selective antagonist of α_1-adrenergic receptors. In our studies prazosin shifted the concentration-response curve for phenylephrine during the luteal phase, follicular phase and in early pregnancy stages to the right in a parallel manner without depressing the maximal response, indicating competitive inhibition. Furthermore, the slope of the Schild plot was not significantly different from unity thus indicating that the antagonism is competitive. The pA scale as described by Schild [77] is used to measure antagonist affinity. The pA_2 value for prazosin was found to be 8.98 in the luteal phase, 9.04 in the follicular phase and 9.10 in the early pregnancy. These values indicate a high affinity of prazosin for α_1 -adrenergic receptors in the isolated porcine uterine artery during the luteal phase, follicular phase, and in early pregnancy. Since antagonists bind to the receptor but do not activate it, the pA_2 value for an antag-

onist in blocking the response to an agonist should be an accurate indication of it's affinity in binding to the receptor [44]. The dissociation constant of prazosin at α_1-adrenergic receptors is typically between 1 and 10nM [73]. The dissociation constant in this study was found to be in the acceptable range. Prazosin was also found to be highly potent(K_i=1.10nM) in competing for uterine arterial binding during estrous cycle and early pregnant gilts [70]. The pA_2 values for prazosin obtained in this study correlate well with the pA_2 values obtained in the other vascular smooth muscle [2], [39]. Agarwal et al. [2] reported that there is a 100 -fold range in prazosin affinity for α-adrenergic receptors. pA_2 Values for prazosin obtained in porcine uterine artery in three different stages of the reproductive cycle are not significantly different ($p > 0.05$). This finding provides evidence that phenylephrine produces contraction of the uterine artery in these three different phases by acting on the same type of receptor.

Determination of the Agonist Dissociation Constant

The validity of the determination of K_A undertaken for phenylephrine depends on the acceptance of the theroetical assumptions of receptor agonist interactions following irreversible receptor inactivation as outlined by Furchgott and Bursztyn [26]. K_A for phenylephrine in the late pregnant ovine uterine artery is reported to be 2.5 x 10^{-6}M [39]. In our present study we obtained a mean K_A value of 6.5 $\pm$ 1.8 x 10^{-6}M in the luteal phase, a K_A value of 3.7 $\pm$ 0.7 x 10^{-6}M in the follicular phase and a K_A value of 4.4 $\pm$ 1.7 x 10^{-6}M in early pregnancy. The K_A values in the present study compare favorably with the values obtained in ovine late pregnant uterine artery, indicating that the same type of α-adrenergic receptors mediate the contractile response in ovine and porcine uterine artery. The results obtained in

the present study indicate that although different concentrations of dibenamine were required to inactivate a fraction of the receptors in the luteal phase, follicular phase and early pregnancy, the affinity of the agonist was the same in all three stages.

Involvement of α_2-Adrenergic Receptors

The porcine uterine artery has been reported to possess both α_1-and α_2-adrenergic receptors [32]. The contractile response to α_2-adrenergic receptor activation has been demonstrated in a few resistance vessels [63]. However, in our present study, medetomidine a selective α_2-adrenergic agonist failed to produce any significant contractile response. The possible reason for the lack of a significant contractile response is that the signal transduction pathways that are normally activated by α_2- adrenergic receptors are not present [61]. Ford et al. [24], using an *in vitro* perfusion technique, have shown in the pig uterine artery from pregnancy that α_2-adrenergic receptors are involved in maintaining tone rather than in evoking the contraction.

The results obtained in our present study are comparable with that obtained in the rabbit uterus. Rabbit uterus contains both α_1-and α_2-adrenergic receptors but the contractile response observed is due to α_1-adrenergic receptors with no functional response resulting from activating α_2-adrenergic receptors [37].

Subclassification of α_1-Adrenergic Receptors

The proposed subclassification by Minneman [54] of α_1-adrenergic receptors into α_{1A}- and α_{1B}- adrenergic receptor subtypes is based on sensitivity to selective antagonists, such as CEC, WB4101, and dependency on extracellular calcium. It has been indicated that the α_{1B}-adrenergic receptor subtype is selectively susceptible to

CEC and is less sensitive to WB4101. The α_{1A}-adrenergic receptor subtype is more sensitive to WB4101 and is less susceptible to CEC. Thus, WB4101 has been indicated to interact differently with CEC sensitive and CEC insensitive α_1-adrenergic receptor subtypes.

We have attempted to subclassify α_1-adrenergic receptors in porcine uterine artery in the luteal phase, follicular phase and in early pregnancy. Our results show that norepinephrine caused dose-dependent contractions of porcine uterine artery during the estrous cycle and in early pregnant pigs. WB4101, an α_{1A} selective antagonist, inhibited uterine artery responses to norepinephrine during estrous cycle and in early pregnancy. The K_B for WB4101 in this study of uterine artery during estrous cycle and in early pregnancy stages correlate with the values reported in other tissues, like renal and mesenteric arteries of rat [35] and rat aorta [65]. Based on the affinity to WB4101, contractile responses to norepinephrine in this present study are mediated by α_{1A}- adrenergic receptors.

It has been suggested that α_1-adrenergic receptor subtypes respond differently to CEC, and thus CEC can be an important pharmacological tool in subclassifying α_1-adrenergic receptors. In this study pretreatment with CEC 5 x 10^{-5} M shifted the concentration-response curve to norepinephrine to the right, and reduced the maximal response. These results indicate that arterial rings of porcine uterine artery during the estrous cycle and early pregnancy are sensitive to a proposed α_{1B}-adrenergic receptor antagonist, CEC. Increasing the concentration of CEC to 1 x 10^{-4}M did not suppress the maximal response to norepinephrine any further than did 5 x 10^{-5}M. Chlorethylclonidine has been used to discriminate the two receptor subtypes by many authors. For example, CEC 5 x 10^{-5}M had no effect on norepinephrine induced

contractions of the dog mesenteric artery while the same concentration of CEC shifted the norepinephrine dose-response curve in the dog carotid artery to the right [57]. Studies from radioligand binding by Eugenia et al. [18] also suggest that CEC is more sensitive in tissues where the α_{1B}-adrenergic receptor subtype is predominant, as compared to the α_{1A}-adrenergic receptor subtype. It has been indicated by these authors [18] that CEC inactivated different proportions of α_1-adrenergic receptor in rat liver cells and rabbit aorta. In rat liver cells (which possess predominantly α_{1B}-adrenergic receptors) 70%-80% of α_1-adrenergic receptors were inhibited by CEC. Whereas, rabbit aorta (which possess predominantly α_{1A}-adrenergic receptors) only 30%-40% of α_1-adrenergic receptors were inhibited by CEC. Based on the sensitivity to CEC in our present study on the porcine uterine arterial rings, our results indicate the presence of α_{1B}-adrenergic receptor subtype.

The literature to-date suggests that α_1-adrenergic receptor subtypes utilize different sources of calcium. The α_{1A}-subtype has been suggested to be dependent on extracellular calcium in mediating the contractile response, whereas the α_{1B}-subtype causes the release of intracellular calcium. In this study nifedipine (a calcium channel blocker) significantly shifted the concentration-response curve to norepinephrine to the right only in the follicular phase. The maximum response to norepinephrine in the presence of nifedipine was depressed significantly in the luteal phase, follicular phase, and in early pregnancy stages. This observation suggests that the contractile response to norepinephrine is not totally dependent on extracellular calcium. These findings indicate that the contractile response to norepinephrine is not mediated by a single receptor, but by a mixture of receptors. The results presented here suggest that both α_{1A}-and α_{1B}- adrenergic receptor subtypes exist in pig uterine artery during

estrous cycle and in early pregnancy stages. Similar observations have been obtained in other tissues which are known to contain both subtypes, such as the portal vein and mesenteric artery of the rat[35].

Analysis of Disposition Mechanism for Norepinephrine

The MAO inhibitor iproniazid did not produce any significant change in the concentration-response relationship to norepinephrine. The potentiating factor obtained for iproniazid a MAO inhibitor was not significantly different from unity ($p > 0.05$), suggesting that this enzyme does not have an important role in the uterine artery during the estrous cycle and early pregnancy for terminating the action of norepinephrine. Similar observaions have been made by Griesemer et al. [30] who indicated that inhibition of MAO with iproniazid did not potentiate the actions of exogenous noradrenaline on the cat nicitating membrane. Furthermore, Hertting and Axelrod [36] reported that MAO seems to be of little importance in the metabolism of ^{3}H-noradrenaline after the release of the labelled catecholamine from sympathetic nerve endings following sympathetic nerve stimulation. Thus, there is little evidence from our experiments or from the literature that MAO is an important mode of disposition for norepinephrine in the blood vessel wall.

The role of COMT in our present study was assessed by the ability of tropolone to modify the contraction to norepinephrine. The COMT inhibitor, tropolone, did not produce any significant change in the concentration-response relationship ($p > 0.05$) to norepinephrine. The potentiating effect of tropolone on the catecholamine (norepinephrine, epinephrine, and α-methylnorepinephrine)-response of rat aortic strip has been demonstrated [51]. The potentiating factor obtained for tropolone in the

present study was not significantly different from unity. Thus, our results suggest that COMT does not represent an important mode of disposition for norepinephrine in porcine uterine artery.

The neuronal uptake (uptake-1) inhibitor cocaine in this study made a significant change in the concentration-response relationship to norepinephrine in the luteal phase, follicular phase as well as in the early pregnant porcine uterine artery ($p< 0.01$). The potentiating factor for cocaine was significantly different from unity suggesting the importance of uptake-1 in the porcine uterine artery. Cocaine has also been reported to potentiate the response to norepinephrine on the rabbit aorta [25]. Cocaine has also been reported to increase the sensitivity to norepinephrine in the rat atria [59]. It has been suggested that cocaine potentiates the response to norepinephrine at least in part by blocking neuronal uptake.

Extraneuronal uptake is a process which involves the removal of the transmitter from the α-adrenergic receptor region. The importance of extraneuronal uptake is suggested by the magnitude of the potentiation of the response of arteries to norepinephrine after blockade of this uptake system. In this study we observed that the uptake-2 blocker corticosterone produced a significant change in the concentration-response relationship to norepinephrine during the estrous cycle and in early pregnancy stages ($p< 0.01$). The potentiating factor obtained for corticosterone was significantly different from unity. Thus, our results indicate that both uptake-1 and uptake-2 play an important role in the disposition of norepinephrine in the wall of the porcine uterine artery in the estrous cycle and in early pregnancy stages. Extraneuronal uptake has also been identified in trachealis smooth muscle cells of cat and rat and in the dog coronary artery [64], as well as in a a cell line derived from cancer

cells of human renal tubules (Caki- cells) [76].

In summary, our results indicate that there is no difference in the affinity of either prazosin or phenylephrine for the α-adrenergic receptor in the porcine uterine artery in the luteal phase, follicular phase, and in early pregnancy. The results also indicate the presence of both the α_{1A}- and α_{1B}-adrenergic receptor subtypes in the luteal phase, follicular phase and in early pregnancy. Based on the results obtained from the study of disposition mechanisms, we conclude that both uptake-1 and uptake-2 play an important role in reducing the concentration of norepinephrine at the α_1-adrenergic receptor in the porcine uterine artery in the luteal phase, follicular phase and in early pregnancy.

For future work, we suggest the use of radioligand binding assays to determine which α-adrenergic receptor subtype is predominant in the porcine uterine artery. Also, we suggest extending our studies to other stages of pregnancy, including mid-pregnancy and late pregnancy stages.

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