

OBSERVATIONS OF IDAZOXAN AND XYLAZINE ON THE MYOMETRIAL
RESPONSE OF THE NORMAL, CYCLING VIRGIN RAT *IN VITRO*

by

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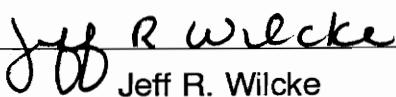
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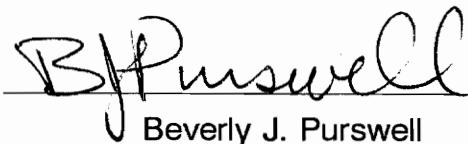
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(ABSTRACT)

The aim of this study was to determine the contractile responses of normal virgin rat uterine smooth muscle to the α_2 adrenergic agonist, xylazine HCl, in the presence or absence of the selective α_2 adrenoceptor blocker, idazoxan HCl. Sections of full thickness uterus measuring $5 \times 1 \times 1$ mm taken from mature, virgin Sprague-Dawley rats were used in isolated tissue baths containing 37°C Krebs-bicarbonate solution, and continually aerated with 95% O_2 and 5% CO_2 . Following stabilization of spontaneous contractions, the tissues were exposed to either no idazoxan (control), 10^{-5} M idazoxan (low), 10^{-4} M idazoxan (medium), or 10^{-3} M idazoxan (high). Five minutes later, xylazine was added to all baths in a cumulative manner at quarter log increments from 1×10^{-5} through 1×10^{-3} M. The % response in peak developed tension and effective concentration resulting in a 50% response (EC_{50}) for the four treatment groups were examined. Results indicated that xylazine alone, at

concentrations greater than 1×10^{-4} M, caused a significant negative inotropic response. Pre-treatment with idazoxan at a concentration greater than 10^{-4} M enhanced the negative inotropic effect of xylazine in a dose-dependent manner. The mechanism of this synergism is unknown but is proposed to be a local anesthetic action due to sodium channel blockade.

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CHAPTER I

INTRODUCTION

A. Principal Aims

Numerous researchers have attempted to quantitate and determine the function of the adrenergic receptor population within the uterus in a variety of species. While some work has been done in non-pregnant, non-hormonally treated, sexually mature females of other species, the function of α_2 adrenoceptors in rat myometrium has not been fully determined.

The aim of this study is to determine the contractile responses of normal virgin rat uterine smooth muscle to the α_2 adrenoceptor agonist, xylazine HCl, in the presence or absence of the selective α_2 adrenoceptor blocker, idazoxan HCl.

B. Literature Review

1. Anatomy of the uterus

The uterus is a hollow, muscular, bicornate organ of reproduction found only in the mammalian female.¹⁻⁴ Positioned in the caudal abdominal cavity, the uterus consists of a cervix, a body and two uterine horns which extend to the

ovaries via the uterine tubes.¹⁻⁴ In most species the two uterine horns diverge from a singular uterine body.¹ (**Figure 1**) The body of the uterus joins the muscular cervix, which serves to protect the uterine environment from pathogens. The size, shape, and proportions of the uterine horns relative to the body vary among species. (**Figures 2, 3**)

Histologically, the uterine wall is composed of three distinct layers: serosal, muscular, and mucosal.² (**Figure 4**) The serosal layer, or perimetrium, is outermost and covers the uterus with reflected visceral peritoneum. The muscular layer, or myometrium, is composed of 3 layers.^{2,4-6} Between the two layers of longitudinal and circular smooth muscle is a prominent third layer of blood vessels, lymphatics, and fine nerve fibers.^{1,2,5,7-9} The mucosa, or endometrium, provides a surface for the fertilized ovum to attach and develop a placenta for growth of the embryo and fetus.^{1,4}

The uterus receives the fertilized ovum and provides the conceptus with nutrients and protection until parturition.^{1,2,4} During parturition, strong rhythmic contractions of the myometrium serve to expel the fetus and then complete placental separation. If myometrial contractions begin before the fetus has fully developed the outcome is abortion, and death, of the fetus.¹

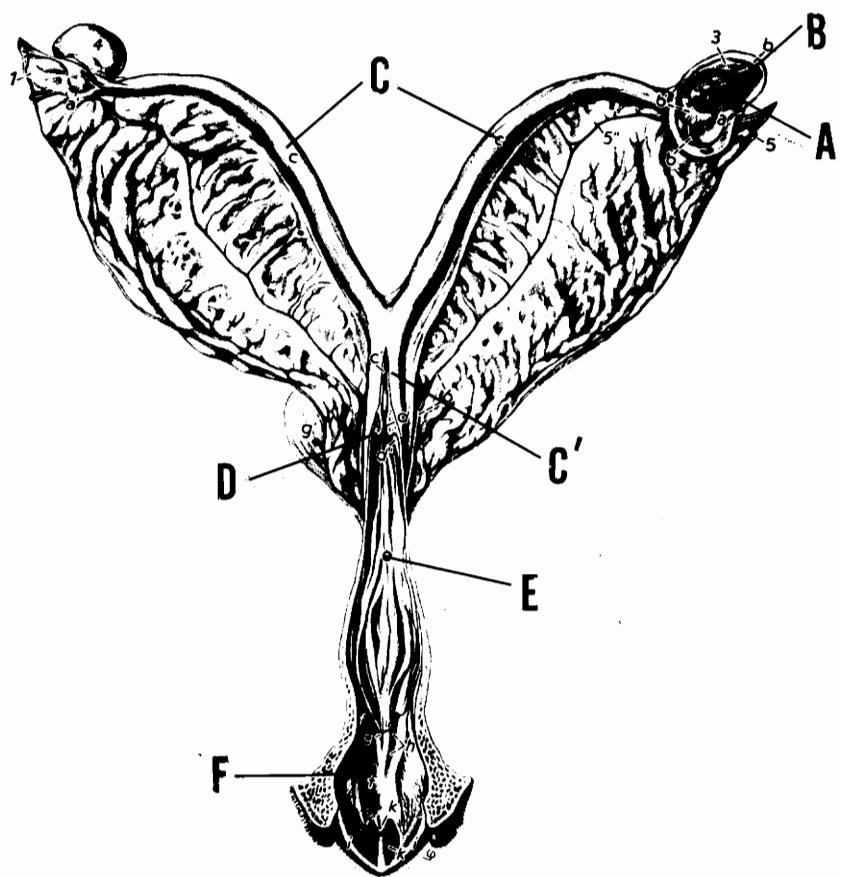


Figure 1: The female reproductive tract. a Right ovary; b Uterine tube; c Uterine horn; c' Body of uterus; d Cervix; e Vagina; f Vestibule (From: Schummer A, Nickel, R, Sack WO. *The Viscera of the Domestic Mammals*. 2nd Revised ed. New York: Springer-Verlag, 1979, p 372.)

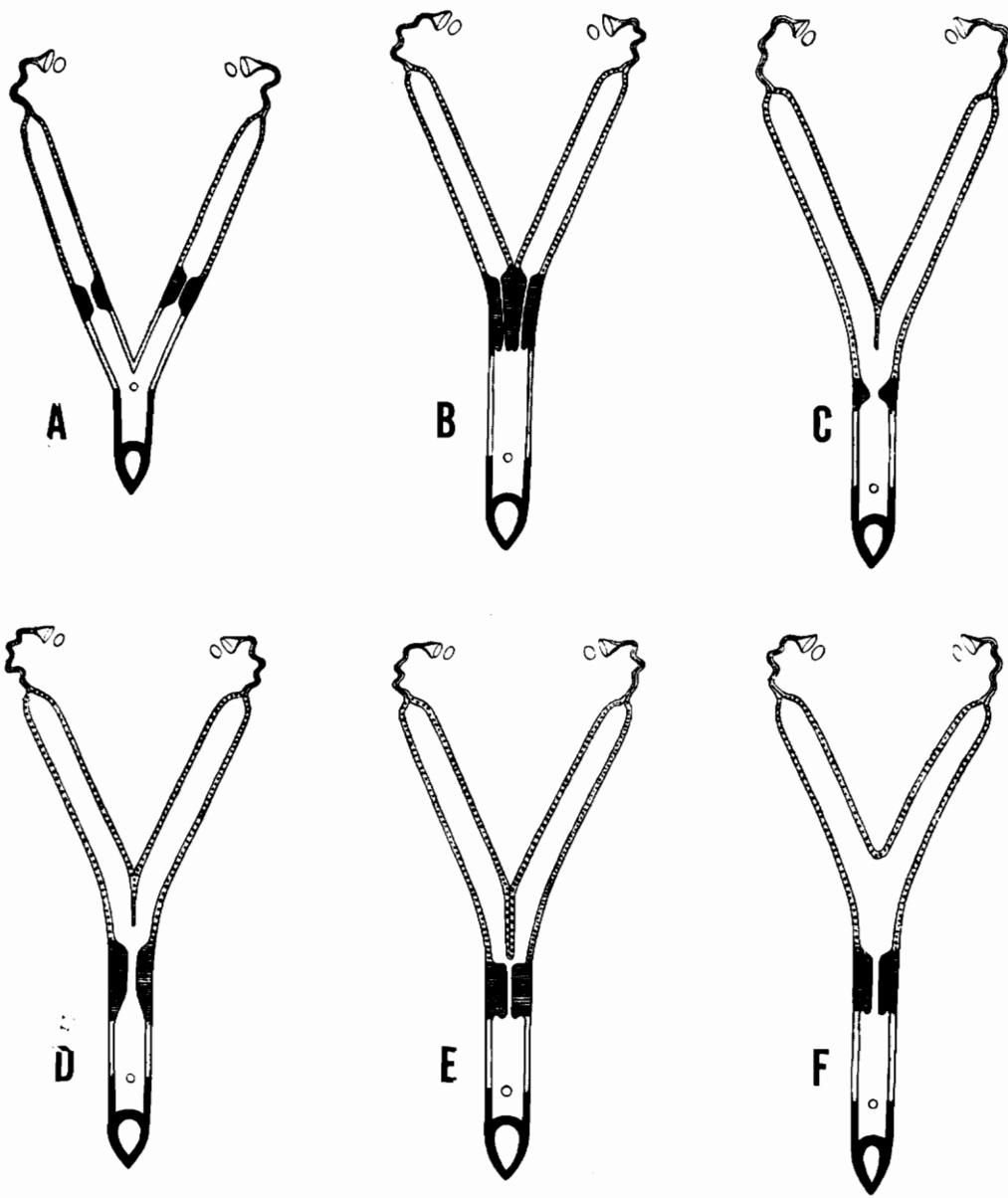


Figure 2: Comparative anatomy of the reproductive tract of domestic mammals.

A Prototypical female genital tract; **B** Rabbit; **C** Dog; **D** Pig; **E** Cow; **F** Horse

(From: Schummer A, Nickel, R, Sack WO. *The Viscera of the Domestic Mammals*. 2nd Revised ed. New York: Springer-Verlag, 1979, p 356.)

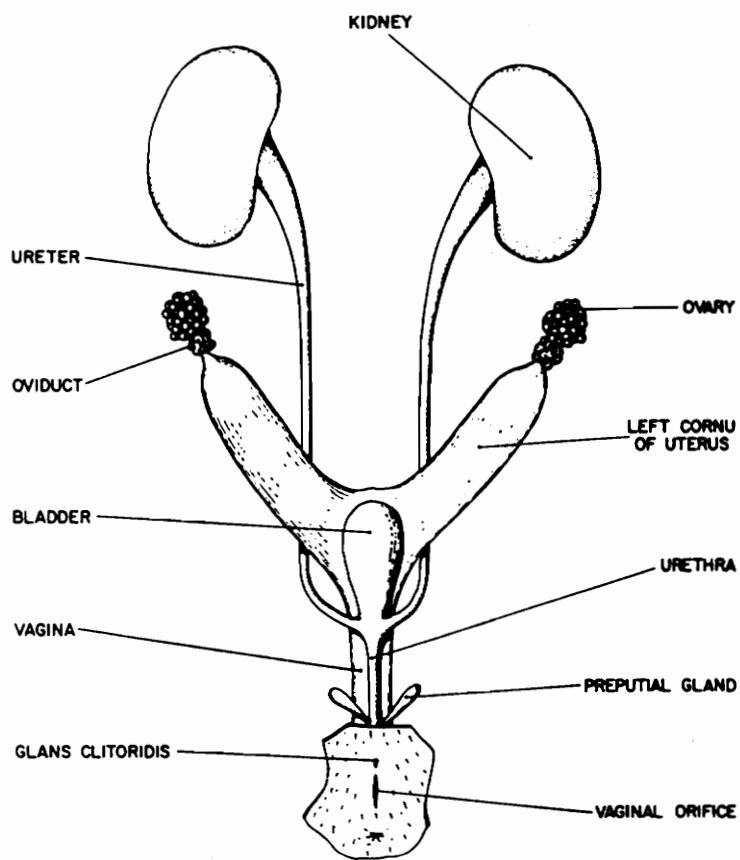


Figure 3: The reproductive tract of the rat. (From: Bivin WS, et al.: In Baker HJ, et al. (eds): *Biology of the Rat*, New York, 1979, p 93.)

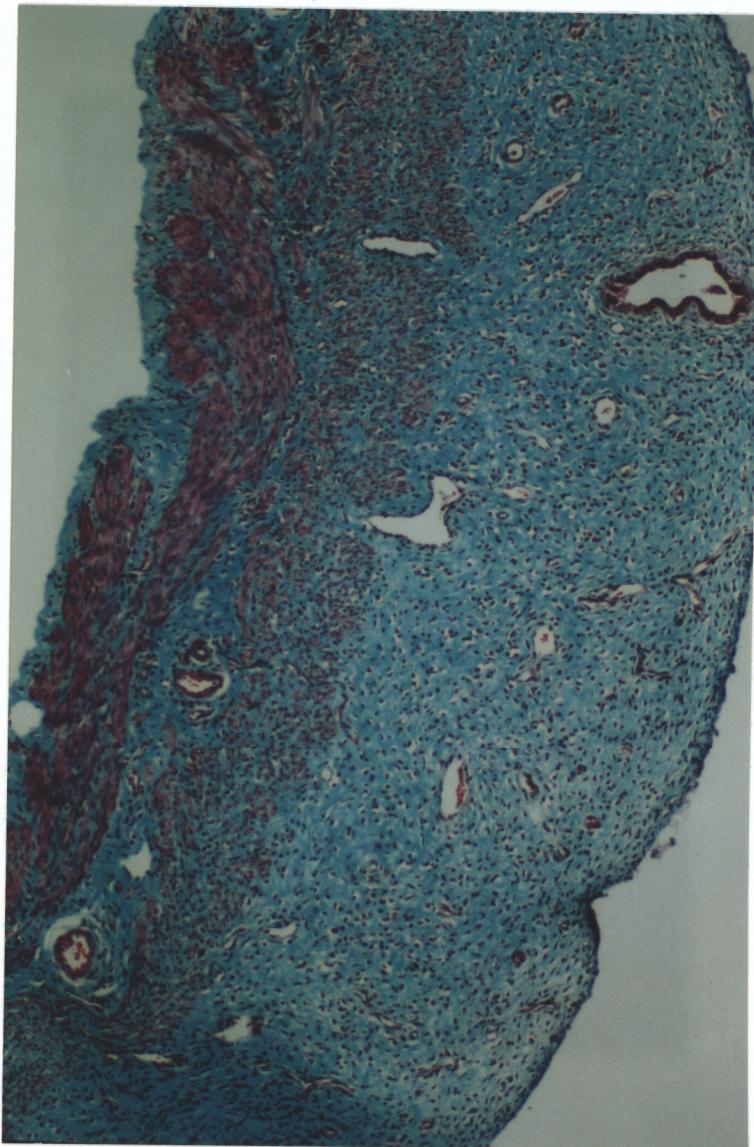


Figure 4: Masson's Trichrome stain of a strip of virgin rat myometrium. The endometrial surface is to the left; the serosa is to the right. The smooth muscle stains blue.

2. Anatomy and physiology of the autonomic nervous system

The autonomic nervous system consists of two broad divisions: the parasympathetic, or cholinergic, and the sympathetic, or adrenergic.¹⁰⁻¹⁴ There is a continuous autonomic balance of parasympathetic and sympathetic tone at all times.^{10,12,14} Tissues and organs receiving autonomic innervation contain receptors for both systems.^{10,11,15}

Tissues receiving parasympathetic innervation possess receptors which bind acetylcholine (Ach), a peripheral neurotransmitter released from parasympathetic nerve terminals and preganglionic sympathetic neurons.¹⁴ When an action potential travels down a parasympathetic nerve fiber and reaches the nerve terminal, Ach is released into the synapse. Acetylcholine then binds Ach-specific receptors in the membrane of the post-synaptic cell. If the post-synaptic cell is another neuron, the action potential is propagated. When the post-synaptic cell is a muscle cell, Ach binding to the cell membrane results in changes necessary for initiation of muscle contraction. Like most naturally occurring neurotransmitters, Ach is rapidly biotransformed at the synapse and the metabolites recycled by the presynaptic neuron to form more neurotransmitter.^{11-14,16,17}

The sympathetic nervous system is more complex than the parasympathetic. The number of possible receptor types within the sympathetic system results in many different neurotransmitters for these receptor sites.¹⁸ Adrener-

gic receptor types are divided and repeatedly sub-divided in attempts to accurately describe specific receptors.¹⁹ Langley first proposed the theory of receptors,^{14,20,21} but it was 40 years later that Ahlquist actually described two pharmacologically distinct types of adrenergic receptors, which he temporarily named alpha (α) and beta (β) receptors.²¹ These original "temporary" names have persisted into today's modern nomenclature.

Later others demonstrated subtypes of both α ²² and β ²³ adrenoceptors. It is presently accepted that the adrenergic receptor is widely variable in type and includes α and β subtypes α_1 , α_2 , β_1 , and β_2 .^{14,15,24-26} Adrenergic receptor sub-classes have also been identified for other neurotransmitters such as dopamine, 5-hydroxytryptamine, etc.^{26,27}

The three most common adrenergic neurotransmitters are 1) dopamine, found primarily within the central nervous system, 2) epinephrine, released from the adrenal medulla into the general circulation at times of stress, and 3) norepinephrine (NE), which is released from postganglionic sympathetic neurons terminating in the periphery; NE is also a major neurotransmitter within the CNS.^{10,12-14,28} Norepinephrine, a naturally occurring adrenoceptor agonist, binds α_1 , α_2 , β_1 , and β_2 receptors.^{29,30} The relative populations of these receptors in a tissue determines the response to NE. Activation of the α_1 adrenoceptor stimulates a phosphodiesterase to split phosphatidyl inositol into two messengers: inositol triphosphate (IP₃) which acts on Ca²⁺ release from the

sarcoplasmic reticulum and 1,2-diacylglycerol (1,2-DG) which activates protein kinase C and promotes a sustained contraction.³¹ (**Figure 5**)

The α_2 adrenoceptor type is less well defined than the α_1 adrenoceptor, though a hypothetical model has recently been proposed.²⁰ The classical description of α_2 adrenoceptors is based on the anatomical location of the α adrenoceptor, ie: presynaptic or postsynaptic.^{19,27,32} This anatomical description defines α_2 adrenoceptors as pre-synaptic inhibitory α adrenoceptors.¹⁵

Activation of this presynaptic α adrenoceptor serves as a negative feedback mechanism for NE release by inhibiting the release of additional NE.^{27,33-37} (**Figure 6**) Once released into the synaptic cleft NE binds with postsynaptic NE specific receptors. The receptor-NE complex alters the permeability of the post-synaptic cell to sodium, potassium, and calcium ions, allowing the action potential to be propagated in the CNS, or muscle contraction to be initiated in the periphery.^{27,38} As long as the inhibitory effect of α_2 adrenoceptor stimulation remains, action potentials reaching the pre-synaptic nerve terminal do not result in release of the neurotransmitter.^{15,19,27,33,40,41} Normal NE metabolism and reuptake at the synapse rapidly reduces the amount of NE available to bind α adrenoceptors, and the pre-synaptic neuron is once again able to release NE in response to an action potential.^{14,15,27,33,39,42}

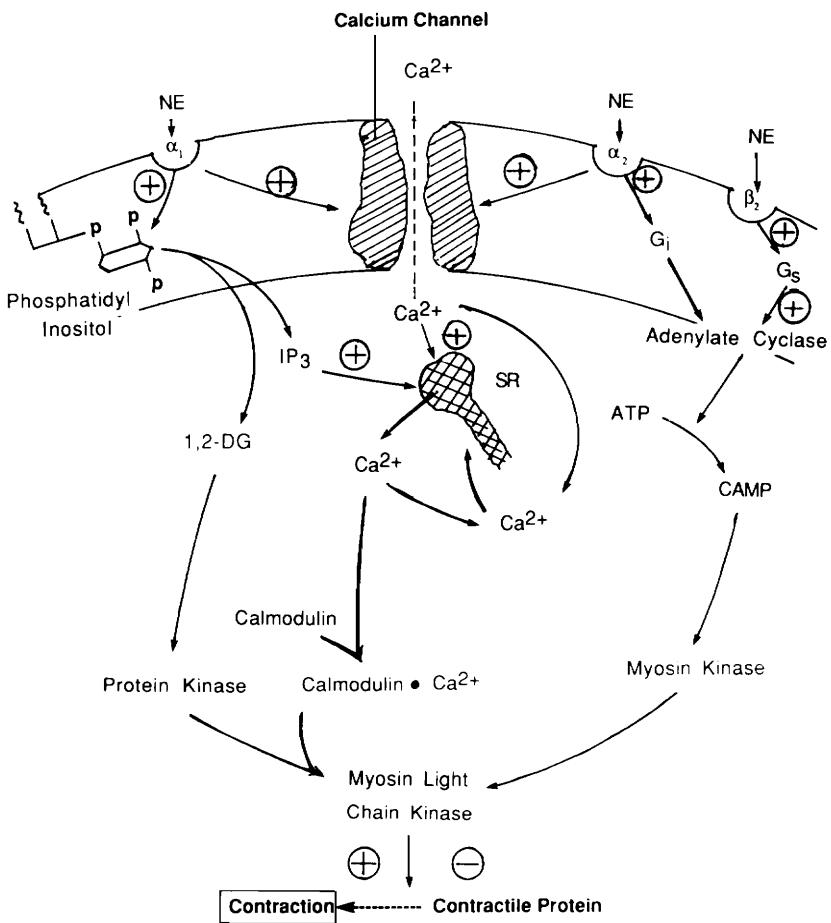


Figure 5: Smooth muscle receptors and the role of receptors in control of muscle contraction.

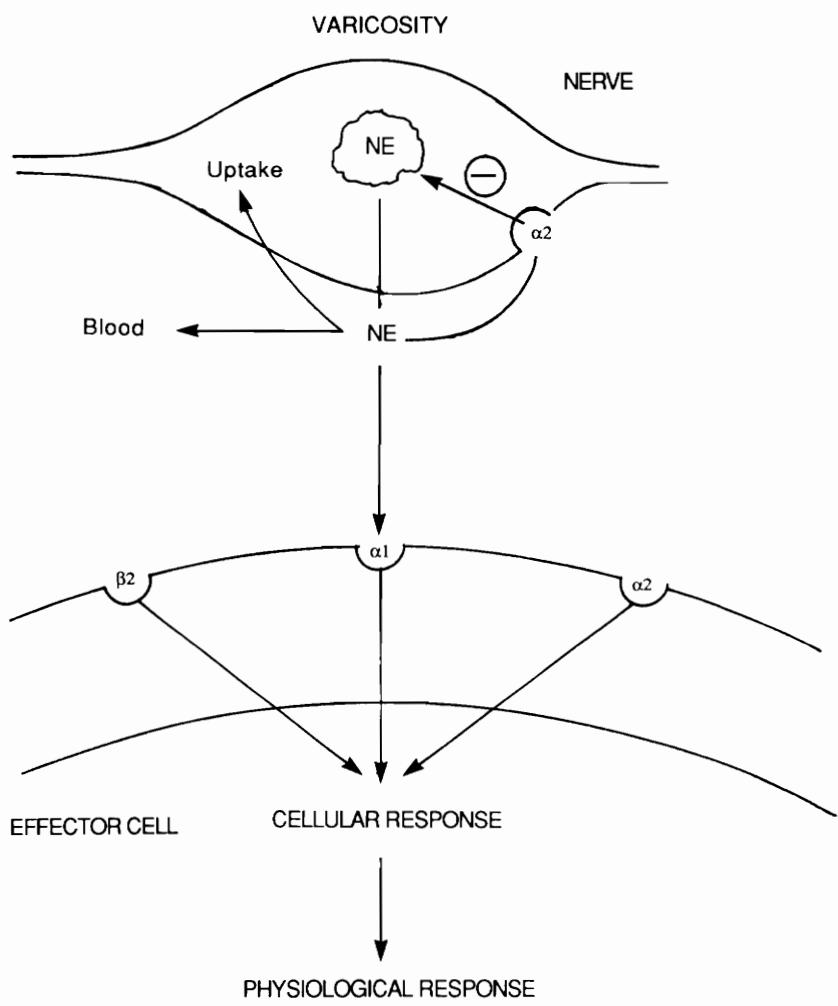


Figure 6: Location of α receptors; sites of NE action.

The anatomical description of presynaptic α_2 vs postsynaptic α_1 , adrenoceptors usually holds true.¹⁵ However, it is now being challenged by pharmacological-binding descriptions.^{15,21,26,43,44} Radioligand studies utilizing tritiated α adrenergic compounds demonstrate that α_1 and α_2 adrenoceptors are not confined to presynaptic and post-synaptic locations.^{15,30,45-47} Alpha₂ adrenoceptors are described pharmacologically as α adrenoceptors possessing a high degree of affinity for compounds such as clonidine, rauwolscine, and yohimbine^{21,40} while α_1 adrenoceptors preferentially bind methoxamine, phenylephrine, and prazosin.^{15,27,40,48}

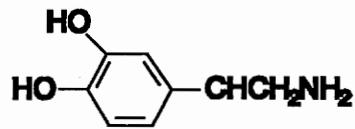
It is now evident that some adrenoceptors on postsynaptic cell membranes selectively bind α_2 specific compounds.^{14,19,41,45,49} While some α_2 adrenoceptors are found on the postsynaptic membrane, many are located on the postsynaptic neuronal membrane outside of the synapse proper. Thus, postsynaptic α_2 adrenoceptors are more correctly described as post-junctional.³² These post-junctional α_2 adrenoceptors are thought to bind not only NE released from the pre-synaptic neuron, but also circulating NE.^{27,30,48} Activation of the postjunctional α_2 adrenoceptor inhibits adenylate cyclase activity necessary for promoting contractions in the smooth muscle. (**Figure 5**)

The molecular structure of a compound determines whether the compound acts as an agonist or antagonist at the receptor as well as the degree of selective binding the compound possesses for a receptor.^{24,32,40,50} An agonist is

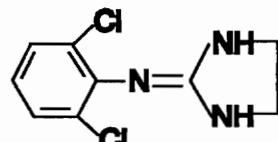
defined as a compound which selectively binds to a receptor and produces a maximal response from that receptor. An antagonist is also highly selective for a receptor and readily binds to the site, which reduces or abolishes the effect of an agonist.

Receptor specificity is not to be confused with receptor affinity. Affinity describes the degree of "tightness" with which a compound binds a receptor.⁶⁰ Receptor specificity relates to how many different types or subtypes of a receptor to which the compound will bind. A highly specific compound will bind to only one or perhaps two receptor subtypes while less specific compounds bind to many subtypes of a receptor class. Many agonist and antagonist compounds have been formulated with varying degrees of specificity and affinity for the different adrenergic receptors.^{51,60}

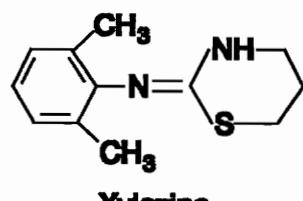
Numerous α -adrenergic active compounds have been formulated and tested *in vitro* in an attempt to develop highly selective α_2 agonists and antagonists.^{9,15,41,43-45,52-55} The α_2 adrenoceptor agonists clonidine^{27,40,56} and xylazine (2-[2,6-dimethylphenylamino]-4H-5,6-dihydro-1,3-thiazin)^{57,65} are thiazide derivatives.^{48,58,59} (**Figure 7**) Both compounds have been studied *in vitro* in isolated tissue preparations, *in vivo* in pithed and intact animals, and have been tritiated for use in radioligand studies.^{27,32,52} Xylazine and clonidine are generally considered to be similar in their pharmacology and mode of action as they are both imidazolines,²⁴ though a recent study demonstrates a significant difference



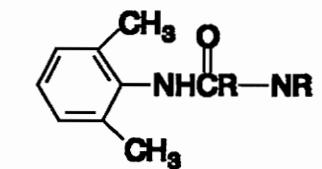
Norepinephrine



Clonidine



Xylazine



Amide Local Anesthetics

Figure 7: Chemical structures of three α_2 adrenoceptor agonists and of the amide local anesthetics.

between the two compounds.⁴⁷

Xylazine is used in veterinary medicine to produce sedation and analgesia in a number of species.^{48,60-63} Though xylazine is considered an α_2 adrenoceptor agonist, there is an initial, transient α_1 effect following intravenous administration.^{64,65} This is manifested clinically as a brief period of hypertension due to α_1 adrenoceptor mediated vasoconstriction.^{57,62,63,66,67} In addition, xylazine also binds other adrenergic and non-adrenergic receptors (α_1 , β , cholinergic, dopaminergic, serotonergic, H₂-histaminergic, and opioid).^{60,66}

The predominant effects of xylazine are attributable to its α_2 adrenoceptor agonist effects as these effects are readily reversed by α_2 adrenoceptors antagonists.^{59,60,68} Central α_2 adrenoceptor stimulation is manifest clinically as sedation, analgesia, and hypotension.^{57,59,61,62,67,69} Peripheral α_2 adrenoceptor effects are annoying in that they are generally considered unwanted side effects. Side effects associated with the use of α_2 adrenoceptor agonists include bradycardia,^{57,62,63,66} hypotension,^{57,63,66} ataxia due to excessive muscle relaxation,^{66,67} gastrointestinal stasis,^{62,63,67} and the risk of abortion when used during the last trimester in some animal species.⁷⁰⁻⁷²

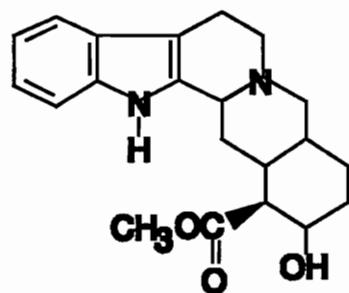
The abortifacient effect of xylazine at clinical dosages has been documented in cattle and is suspect in other species.⁷⁰⁻⁷³ *In vivo* studies in pregnant and non-pregnant food animals demonstrate significant increases in intrauterine pressure^{71,72,74} and electromyographic activity⁶¹ following xylazine administration.

Xylazine induces an increase in spontaneous myometrial contractions when administered to instrumented cattle⁷² and sows.⁷⁵ This effect has been replicated *in vitro* with bovine myometrium in isolated tissue baths.^{76,77} Xylazine induced contractions can be blocked by α_2 adrenoceptor antagonists such as yohimbine and idazoxan, but not by prazosin, a selective α_1 adrenoceptor antagonist.^{74,76}

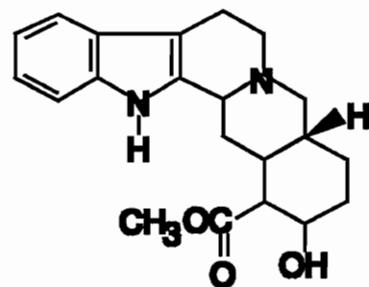
The α_2 antagonists most often employed in *in vitro* studies are rauwolscine, yohimbine, and idazoxan.^{43,48,50} (**Figure 8**) Other more selective compounds have been used in radioligand studies, and though some have subsequently been used in isolated tissue preparations, few have been used *in vivo*.^{50,53} Tolazoline is an α_2 adrenoceptor antagonist often used *in vivo* to reverse the effects of xylazine. However, it lacks sufficient α_2 adrenoceptor specificity to be useful in *in vitro* studies.^{15,59,60}

Rauwolscine was one of the first α adrenoceptor antagonists discovered and is highly specific for α_2 adrenoceptors.^{32,41,50} Yohimbine is also specific for α_2 adrenoceptors,^{29,42,51} though in some studies it demonstrates binding at other receptors, including postsynaptic α_1 adrenoceptors.^{53,77-79}

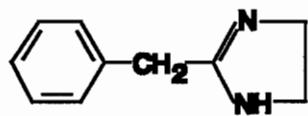
Idazoxan (2-(2-(1,4-benzodioxanyl)-2-imidazoline), an imidazoline, was formulated in the late 1970's and represents a new class of α_2 adrenoceptor antagonists.⁵⁰ It is highly selective for α_2 adrenoceptors and demonstrates no significant *in vitro* binding to other receptor types.^{41,44, 53-55,80} Idazoxan is



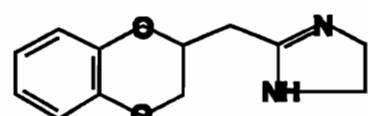
Rauwolscine



Yohimbine



Tolazoline



Idazoxan

Figure 8: Chemical structures of four α_2 adrenoceptor antagonists.

reported to be six times as selective as yohimbine for the α_2 adrenoceptor⁵⁰ and is considered one of the most potent (high receptor affinity) and selective α_2 adrenoceptor antagonists.⁸¹

3. Uterine adrenergic physiology

The uterus receives autonomic innervation from both the parasympathetic and sympathetic nervous systems via the pelvic nerve and hypogastric plexus, respectively.^{8,82,83} In addition, there is a network of fine non-myelinated nerves within the myometrium. This intrinsic innervation controls myometrial contractions and has been described as being "broadly coordinative, slow acting, not sharply localized and non-specific in that the motor response to nervous stimulation is conditioned by the dominant ovarian hormone".² This intrinsic nervous network continues to function normally even after uterine autonomic denervation, provided ovarian hormones remain present.⁸³

Three adrenergic receptor types, α_1 , α_2 , and β_2 adrenoceptors have been identified in the uterus, specifically in the myometrium.^{9,14,46,49,84,85} The most prevalent adrenoceptor in the myometrium is the α receptor.^{66,86-88} Both α_1 and α_2 adrenoceptors are found in the myometrium,^{53,56,86,87} though the number of each type depends on the species,^{70,89-92} gonadal hormones present,^{73,82,86,93} and the muscle layer studied.^{49,84} Radioligand studies demonstrate both α_1 and α_2 adrenoceptors on the plasma membrane of the rat myometrial cells.⁹ Alpha,

adrenoceptors are associated with the contractile response of the myometrium much like that observed in vascular smooth muscle.^{70,86,87,89,94} Although a large population of α_2 adrenoceptors have been identified in the myometrium, their function in the myometrium has not been determined.^{56,66,73,85,92}

The objective of this study was to determine the effect of idazoxan, a highly selective α_2 adrenoceptor antagonist, on the effect of spontaneous uterine contractions following exposure to xylazine HCl, an α_2 adrenoceptor agonist, in vitro.

CHAPTER II

METHODS AND MATERIALS

A. Tissue Preparation

Mature, nulliparous, non-pregnant, female Sprague Dawley rats weighing 200-250 grams were anesthetized by intraperitoneal injection of sodium pentobarbital (30 mg/kg). A ventral midline incision was made and the uterus identified. Clamps were applied to each ovarian plexus and the uterus removed from the uterine ducts to the junction of the horns at the uterine body.

(Figure 9) Upon removal, the uterus was immediately placed in a beaker of room temperature (20° C) Krebs bicarbonate solution. Carbogen (95% O₂/5% CO₂) was continuously bubbled through the solution. The rat was then immediately euthanized with an overdose (200 mg/kg) of sodium pentobarbital IP.

The broad ligament and accompanying blood vessels were removed from the uterine horns and the two horns separated at their junction with the uterine body. Each horn was opened longitudinally along the mesometrial boarder and two pieces measuring 5 mm in length were removed from the fundal end of each uterine horn. **(Figure 9)** The pieces were trimmed to produce strips 1 mm wide by 5 mm long.

Full thickness uterine strips were used due to the difficulty in adequately removing the serosal and endometrial layers without damaging the myometrium. One end of each strip was tied with 3-O silk suture for attachment to an isometric force transducer. The free end was secured in a clamp and submerged in 37° C Krebs bicarbonate solution in an isolated muscle bath.

B. The *in vitro* system

Four water-jacketed, 100 ml isolated muscle baths were used.

(Figure 10) The temperature of the Krebs bicarbonate solution within the baths was maintained at 37 ± 1° C. The Krebs bicarbonate solution used in the baths had the following composition (mM): NaCl 118, KCl 5, MgSO₄ 2, KH₂PO₄ 1, NaHCO₃ 25, CaCl₂ 2, and dextrose 11. The Krebs bicarbonate solution in the muscle bath was continuously aerated with 95% O₂ and 5% CO₂. At rinsing, the baths were drained and refilled with pre-warmed (37°C) Krebs bicarbonate solution by gravity flow.

The uterine strips were suspended in pairs in each bath chamber. Changes in contractile force were measured with a Grass (FT03) isometric force transducer and recorded on a grass polygraph (Model 7D) at a chart speed of 25 mm/sec.

An initial resting tension of 0.25 grams was applied to the muscle strips. Each muscle was allowed at least 30 minutes for stabilization in the muscle

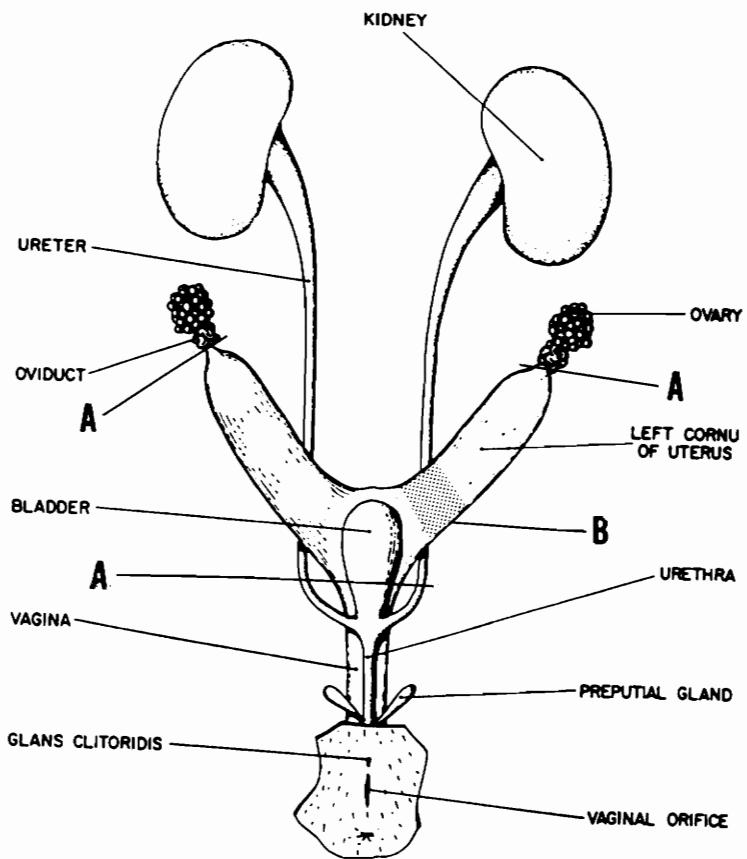


Figure 9: Placement of clamps for removal of uterus (A) and section of uterine horn from which tissue strips were obtained (B).

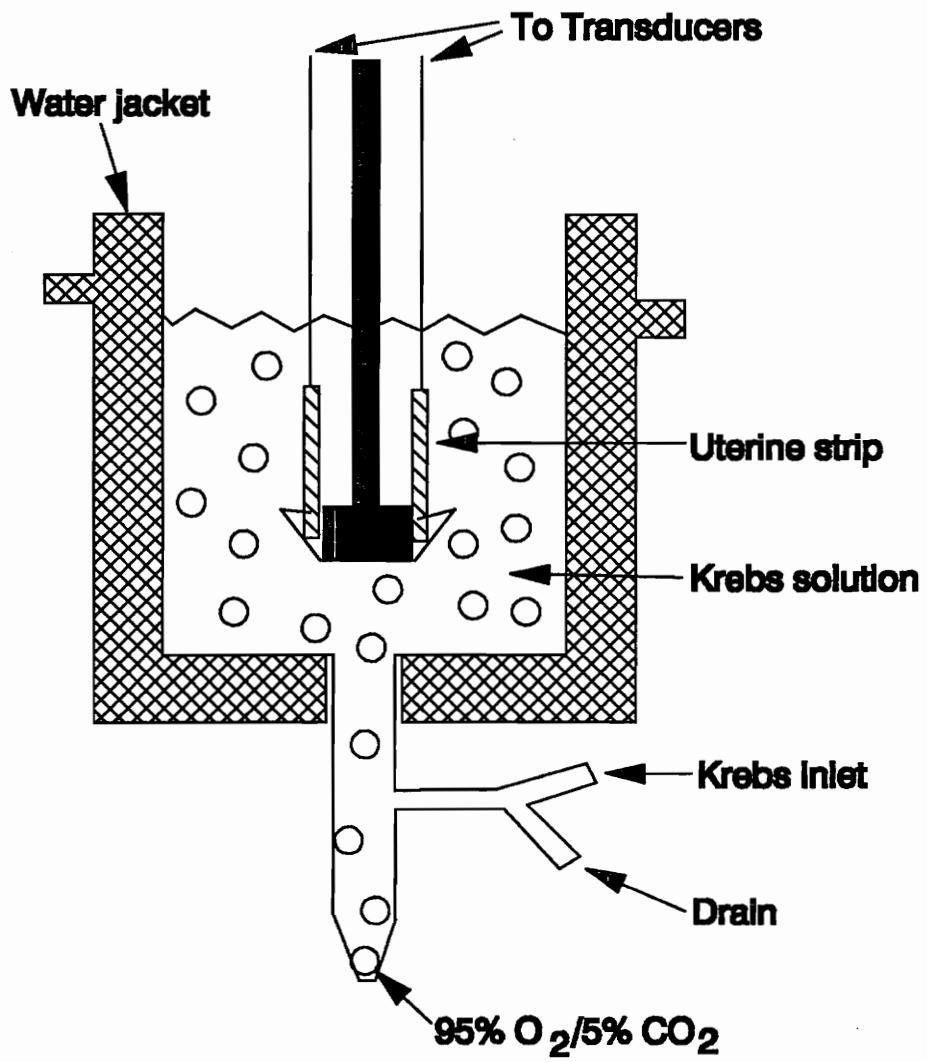


Figure 10: A water bath for use in isolated muscle studies.

bath to develop rhythmic, spontaneous contractions.

All drugs and solutions were made fresh at the start of each day. The drugs used were xylazine HCl (Sigma) and idazoxan HCl (Ricket-Coleman) and dissolved in distilled, de-ionized water.

C. Experimental Design

The effects of idazoxan on responses of myometrium to xylazine were investigated in 70 uterine muscle strips. Muscle strips were grouped into four treatment groups: no idazoxan (control) ($N=19$), 1×10^{-5} M idazoxan (low) ($N=17$), 1×10^{-4} M idazoxan (medium) ($N=17$), and 1×10^{-3} M idazoxan (high) ($N=17$). Concentration response curves to xylazine were obtained by increasing the concentration cumulatively every two minutes.⁹⁵ Nine xylazine concentrations were studied, from 1×10^{-5} M through 1×10^{-3} M inclusive (1×10^{-5} M, 2.5×10^{-5} M, 5×10^{-5} M, etc). Preliminary studies indicated that a period of two minutes was sufficient for the muscle to produce the maximum response. This procedure resulted in reproducible contractile responses to each dose of xylazine. In those experiments examining the effects of idazoxan on the xylazine induced contractile response, idazoxan was added to the bath five minutes before the first dose of xylazine to be tested. Each strip was exposed to only one concentration of the antagonist, either 1×10^{-5} M, 1×10^{-4} M, or

1×10^{-3} M. Two strips of muscle from each run were not exposed to the idazoxan and served as controls.

D. Data Analysis

The contractile response measured in this study was the peak developed tension of the spontaneous contractions. The physiograph was calibrated to 2 grams/cm chart deflection and the height of the contraction was measured using SigmaScan¹ and a digitizer pad². The height was converted to grams using the equation:

$$\text{Grams of developed tension} = \frac{\text{Height (mm)} \times 2 \text{ Grams}}{10 \text{ mm}}$$

A baseline response for each tissue was determined by averaging the developed tension of the contractions in the two minute interval immediately preceding the first addition of xylazine. This baseline value was considered the 100% response by the muscle. All other responses were calculated in a similar manner, compared to the 100% response, and expressed as % response.

The response to xylazine obtained in uterine muscle strips incubated with various concentrations of idazoxan was compared with the response obtained in the untreated muscle strips. To determine whether the xylazine concentration

¹ Sigma-Scan, Version 3.0, Jandel Scientific, Corte Madera, CA

² SummaSketch II, Summagraphics Corp, Seymour, CT.

response curves were significantly displaced by idazoxan, the comparisons were made at the 50% of the maximal response (EC_{50}). This procedure permitted comparison in the dose-response curves among the different muscle strips. The EC_{50} values for individual tissues were determined as follows.

Using PC-Nonlin³, a probit scale was used to determine the line of best fit to the data for individual concentration response curves. The slope and Y-intercept of this line were determined according to Equation 1 using PC-Nonlin.

$$Y = mX + b \quad (Eq. 1)$$

Equation 1 was rearranged to give Equation 2

$$X = \frac{Y - b}{m} \quad (Eq. 2)$$

where m = slope, b = Y-intercept, Y = the probit value for 50% (.50), and X = the corresponding xylazine concentration. Solving for X using the values determined (slope and Y-intercept) gives the xylazine concentration that results in a 50% response by the muscle (EC_{50}). (**Figure 11**)

All values are shown as means \pm SEM. Statistical comparisons were made using one way ANOVA to determine differences among treatment groups for a given xylazine concentration, and within a group across the cumulative concentration response curves. Those xylazine concentrations and treatment

³ PC-Nonlin, Version 3.0, SCI Software, Lexington, KY.

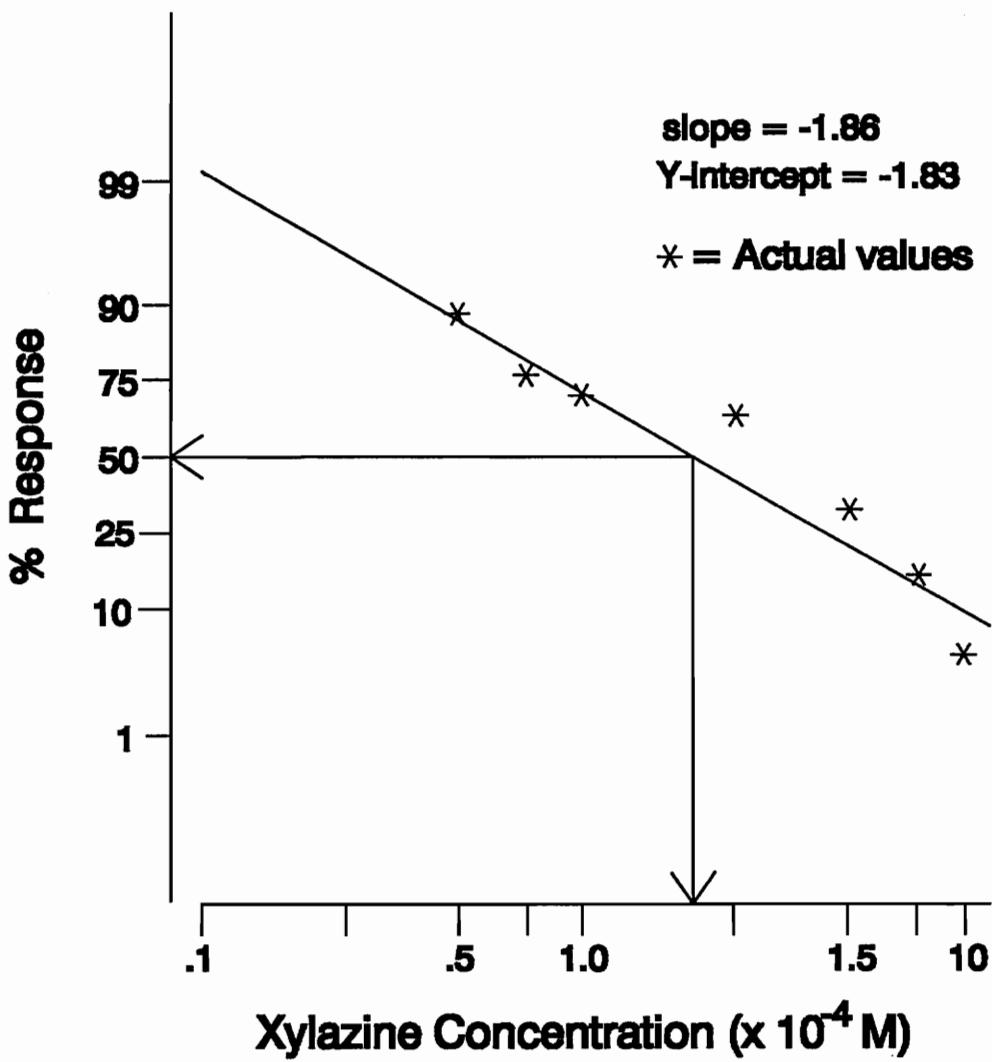


Figure 11: A probit-log plot of data from one muscle sample showing the actual data points and the line of best fit from the EC₅₀ determination for that muscle.

groups demonstrating a significant difference were tested further for differences between groups and between xylazine concentrations within a group using Student's unpaired t-test. The mean EC₅₀ for each treatment was compared to control using Student's unpaired t-test. Differences were considered significant if p < 0.05.⁹⁶

CHAPTER III

RESULTS

No significant differences in the size or initial control contractions of the muscle strips were observed between experimental groups. The uterine muscle strips were suspended so that under their own buoyancy they measured 5 mm in length between attachment points. The uterine strips were previously trimmed to 1 x 1 mm (1 mm² cross-sectional area). The mean tension (\pm SEM) of initial control contractions was 0.677 ± 0.04 grams. It is noteworthy that idazoxan has no significant effect on the initial control contraction of the muscle strips.

Table 1 summarizes the effect of idazoxan on the xylazine-induced contractile response of the virgin rat uterine muscle strips. The values for contractile response before xylazine were taken as 100%. In the control group, there is no significant attenuation of muscle contraction until a xylazine concentration of 5×10^{-4} M in the muscle bath is reached. Pretreatment with a low concentration (1×10^{-5} M) of idazoxan had no effect on the xylazine-induced contractile response in the rat uterine muscle strips. However, it is evident from the data shown in Table 1 that the sensitivity of xylazine-induced attenuation of muscle contraction in rat uterine strips was progressively increased with the concentration of idazoxan pretreatment. High concentrations (1×10^{-3} M) of

Table 1: Percent response (% of baseline response) by uterine tissues receiving only xylazine HCl (control) and uterine tissues exposed to one of three concentration of idazoxan HCl for 5 minutes prior to and during construction of a cumulative concentration response curve to xylazine HCl. Values are expressed as mean \pm SEM.

XYLAZINE CONCENTRATION	IDAZOXAN PRE-TREATMENT (% of Baseline Response)			
	CONTROL (N=19)	LOW (N=17)	MEDIUM (N=17)	HIGH (N=17)
1 X 10^{-5} M	112.73 \pm 7.29	106.52 \pm 9.85	108.19 \pm 4.79	92.79 \pm 4.58
2.5 X 10^{-5} M	111.37 \pm 8.45	110.38 \pm 15.83	101.40 \pm 7.07	93.00 \pm 12.44
5 X 10^{-5} M	108.57 \pm 10.78	107.74 \pm 14.77	96.14 \pm 5.53	* 74.30 \pm 5.01
7.5 X 10^{-5} M	108.41 \pm 12.38	114.67 \pm 19.11	102.94 \pm 14.74	* 60.57 \pm 5.27
1 X 10^{-4} M	109.74 \pm 13.73	101.51 \pm 12.40	95.02 \pm 10.14	* 56.49 \pm 5.93
2.5 X 10^{-4} M	98.07 \pm 11.06	80.82 \pm 11.43	* 73.56 \pm 6.06	* 47.91 \pm 5.04
5 X 10^{-4} M	* 72.44 \pm 12.04	* 62.41 \pm 13.58	* 38.75 \pm 5.40	* 21.82 \pm 5.09
7.5 X 10^{-4} M	* 37.45 \pm 5.28	* 35.98 \pm 7.52	* 12.81 \pm 3.90	* 12.62 \pm 5.01
1 X 10^{-3} M	* 27.39 \pm 8.11	* 16.97 \pm 4.06	* 5.82 \pm 2.50	* 2.50 \pm 2.17

Control: No Idazoxan

Medium: 10^{-4} M Idazoxan

Low: 10^{-5} M Idazoxan

High: 10^{-3} M Idazoxan

*Differs significantly ($p < 0.05$) from baseline response for that treatment.

idazoxan produced a significant leftward shift of the xylazine concentration response curve. (**Figure 12**)

These relationships were further examined by a comparison of concentration response curves at 50% of the maximal response (EC_{50}) among different treatment groups. Figure 13 shows the relationship between EC_{50} and different experimental groups, in the absence and presence of increasing concentrations of idazoxan. The mean value of EC_{50} (\pm SEM) for the untreated control muscle group was $6.3 \pm 1.9 \times 10^{-4}$ M. This value was progressively decreased with increasing idazoxan concentrations. The mean EC_{50} (\pm SEM) for low, medium, and high idazoxan treatment groups were 4.2 ± 0.8 , 2.2 ± 0.2 , and $1.5 \pm 0.3 \times 10^{-4}$ M, respectively. Compared to control, this shift was significant only at the high idazoxan (1×10^{-3} M) concentration level.

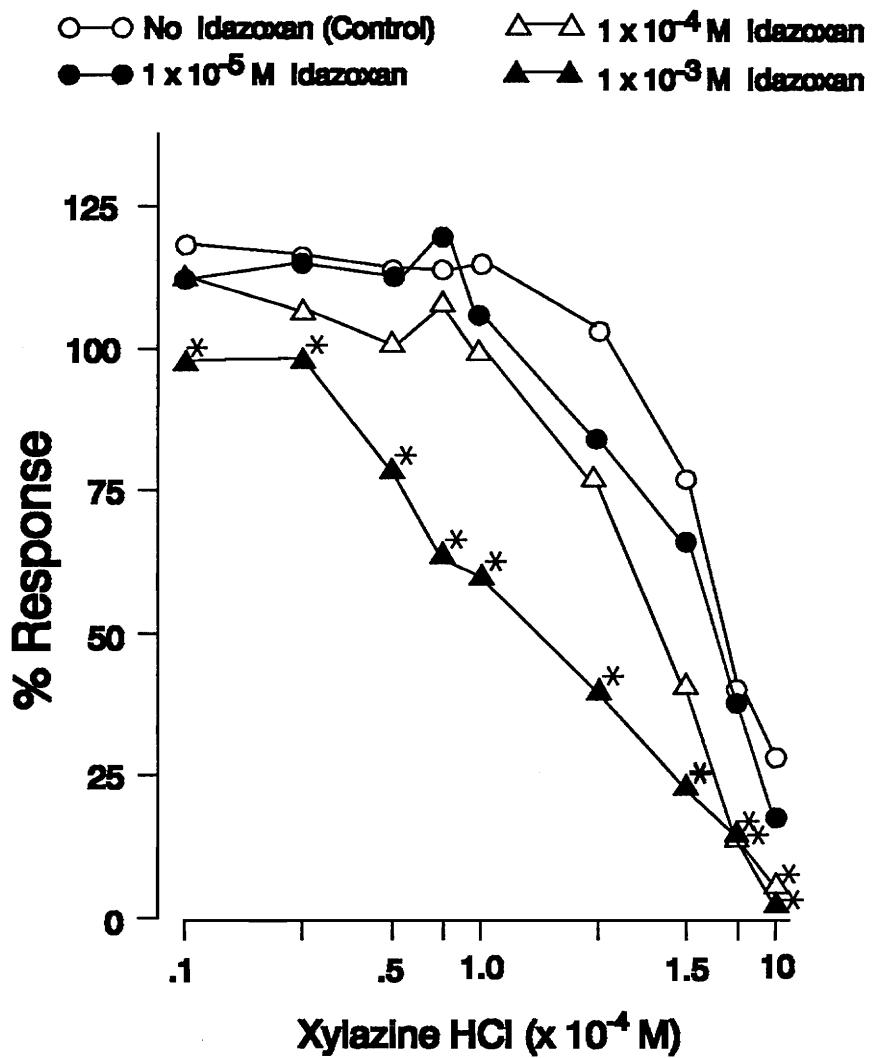


Figure 12: Semi-log plot of the xylazine concentration response curves in virgin rat myometrium in the absence and presence of increasing idazoxan concentrations.

*Differs from control.

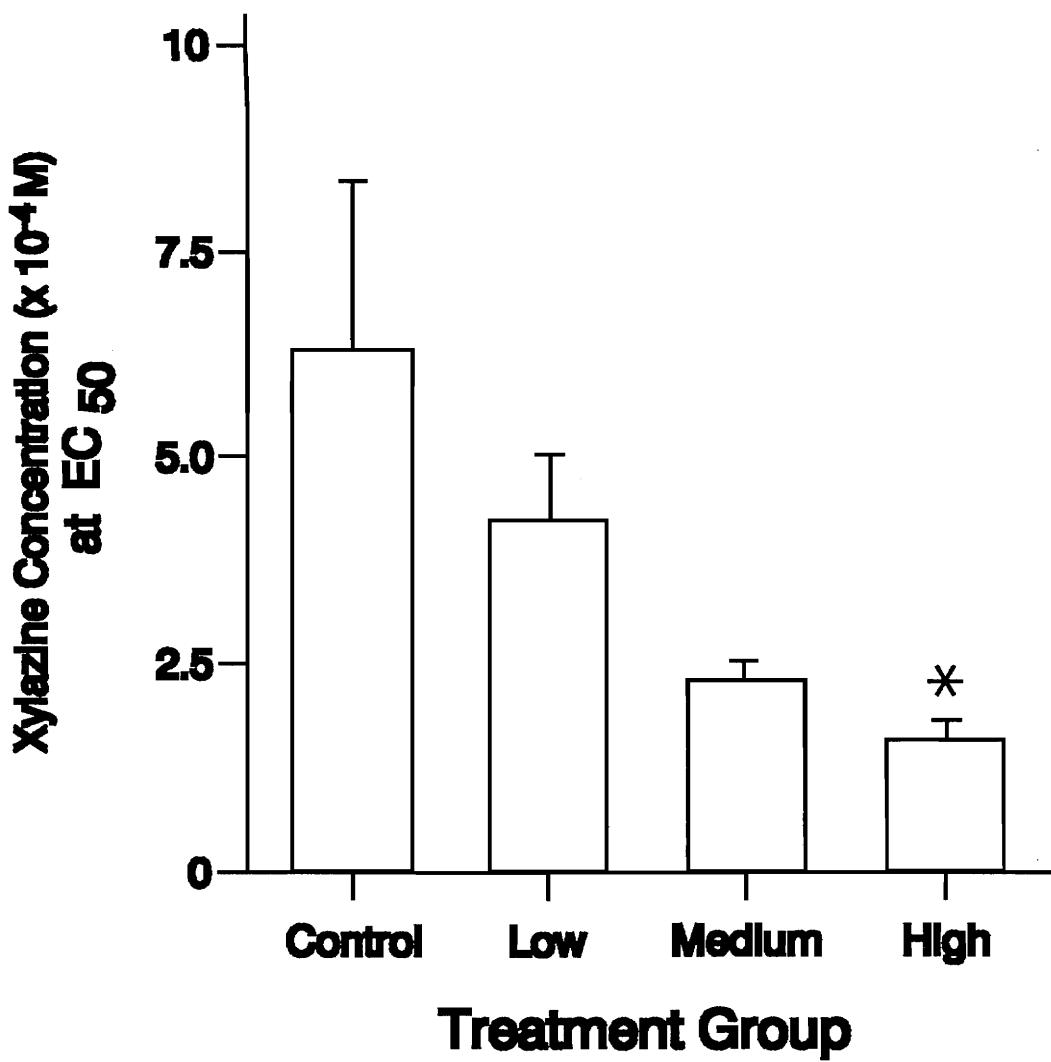


Figure 13: The EC₅₀ (mean \pm SEM) of xylazine in virgin rat myometrium for control and three concentrations of idazoxan.

*Differs from control.

CHAPTER IV

DISCUSSION

This study was undertaken primarily to examine the effects of idazoxan on xylazine induced contractile responses in uterine muscle of virgin rats and evaluate α_2 adrenoceptor action on these muscles. The objective was to apply established isolated muscle preparations for assessing and quantifying the contractile actions of virgin rat uterine muscle to an α_2 adrenoceptor agonist and antagonist. The contractile response to the agonist was defined by concentration response curves and the response following pre-treatment with a specific antagonist was assessed by the shift of EC₅₀.

The most important finding of this study is that this preparation could not elucidate a response attributable to α_2 adrenoceptor function in virgin rat myometrium. Xylazine concentrations greater than 1×10^{-4} M produce a dose-dependent negative inotropic action on uterine muscle of virgin rats and moreover this action was potentiated by the pretreatment of idazoxan. In contrast, Ko et al reported that xylazine increases uterine contractility of bovine myometrium in a dose dependent manner from 10^{-8} to 10^{-6} M concentration.⁷⁶ Similar findings have also been demonstrated in some other species.^{61,67,70-77,84} There is no clear explanation for the observed differences in sensitivity of the contractile response to xylazine.

These differences, however, cannot be ascribed entirely to the ranges of concentrations studied. In our preliminary studies we consistently found no significant positive effect of xylazine on uterine muscle of virgin rats ranging from 10^{-11} to 10^{-4} M. It has been shown that ruminant, in particular the domestic ruminants, are more sensitive to the effects of xylazine than non-ruminants, including the rat.^{59, 60, 65, 67, 95} Species differences in the uterine response to α_2 adrenoceptor agonists are due to differences in the relative number and/or function of α_2 vs α_1 adrenoceptors within the myometrium.^{38, 70, 73, 85-87, 89, 90, 94, 98, 99} It is also of interest that the changes in estrogen and progesterone levels alter the relative number and function of myometrial adrenoceptors.^{86, 88, 98-104} These changes vary substantially among various species^{70, 86, 89, 92, 94, 101, 103} and age of the animal.^{56, 86, 105}

The likelihood that the negative inotropic properties of xylazine can be ascribed to the tachyphylaxis is ruled out by our early pilot studies. The negative inotropic action of xylazine was independent of the duration of exposure to xylazine and the concentration of xylazine to which the muscle strip had been previously exposed. Several possibilities must be considered to explain the negative inotropic effect of xylazine. It is well recognized that xylazine is classified as an α_2 agonist, acting predominantly at α_2 adrenoceptors.^{57, 59, 62, 63} However, xylazine has also been shown to have a local anesthetic action *in vivo* and *in vitro*.^{67, 97, 106} Xylazine and other α_2 agonist share a common chemi-

cal structure with the amide local anesthetics.⁶⁴ (**Figure 7**) The local anesthetic action of xylazine may relate to the direct blockade of voltage-sensitive sodium channels.^{78,107} It has been suggested that xylazine, lidocaine, or other local anesthetic agents may obstruct the external opening of the membrane channel, preventing the influx of sodium ions through the channel.¹⁰⁷

Xylazine could also indirectly cause sodium channel blockade when bound to postjunctional α_2 adrenoceptors through inhibition of the intracellular second messenger, cAMP. Alpha₂ adrenoceptor agonists have been shown to stimulate the inhibitory guanine nucleotide protein (G_i), resulting in the inhibition of cAMP production thus lowered the state of activation of the cAMP dependant protein kinase and inactivation of various voltage-sensitive membrane channels, including sodium and calcium.^{6,50,66,85,92,108-112} Either mechanism would effectively prevent the sodium influx necessary for depolarization of the postsynaptic cell.

Visceral smooth muscle is formed by sheets of muscle cells which undergo spontaneous contractions.^{113,114} It is self excitatory and action potentials occur without an extrinsic stimulus.⁵ Plateaus in smooth muscle action potentials are common and last up to 30 seconds.^{91,104,113} Uterine smooth muscle is characterized by a high degree of spontaneous activity.^{5,82,84,90,91,104,}

^{113,115-117} In uterine smooth muscle, movement of sodium ions is the primary determinant in depolarization.¹¹³

Due to a poorly developed sarcoplasmic reticulum, smooth muscle relies on an extracellular source of calcium for contraction.^{113,118} If xylazine is acting via the G_i protein through binding to the α_2 adrenoceptor, it is likely that the calcium channels in cell membranes are blocked. The inhibitory effect we observed at xylazine concentrations greater than 1×10^{-4} M could therefore be due to a reduction in calcium influx through the voltage-sensitive channels. In addition, inhibition of adenylate cyclase can also activate potassium conductance.¹¹⁹ This would, of course, be expected to influence muscle contraction.

Earlier studies indicated that idazoxan HCl, a selective α_2 adrenoceptor antagonist, reverses the effects of xylazine on the uterus.^{76,84} Ko et al⁷⁶ report that in isolated bovine myometrium, the increase in uterine contractility in response to xylazine is antagonized by idazoxan HCl in a dose dependent manner at idazoxan concentrations of 10^{-8} , 10^{-7} , and 10^{-6} M. A recent report⁴¹ indicates that at concentrations greater than 10^{-6} M idazoxan produces contractions in isolated rat vas deferens. Thus, it was reasonable to expect that the concentration response curve to xylazine to either remain unchanged or to flatten and shift to the right as the concentration of idazoxan was increased. However, in this study we have found that the concentration response curve to xylazine shifted downward and to the left in a dose-dependent manner with increasing concentrations of idazoxan. This dose dependent change in the

response to xylazine by idazoxan indicates that idazoxan potentiates the negative inotropic effect of xylazine on isolated rat myometrium.

These results are consistent with earlier reports that idazoxan is capable of causing uterine smooth muscle relaxation.^{38,82,87,94,98,99} This offers a likely explanation for the downward and leftward shifts in the concentration response curve to xylazine we observed. To date, only one study has investigated the effect of idazoxan alone on an isolated tissue.⁴¹ It should be of course recognized that the present study did not investigate the concentration response curve to idazoxan alone on isolated virgin rat myometrium.

It is interesting to note that both xylazine and idazoxan share a common chemical structure with histamine (β -imidazolylethylamine) in that each of the three compounds contain an imidazole ring. It has been shown that histamine induces myometrial relaxation in the rat.¹²⁰ While xylazine does not display histiminoergic activity at clinical doses, it is conceivable that at high concentrations ($> 1 \times 10^{-4}$ M), there is significant binding of xylazine to H₂-histamine receptors. Likewise, idazoxan has not been reported to bind appreciably to receptors other than α_2 adrenoceptors at concentrations corresponding to clinical doses. The concentrations in this study which are associated with a negative inotropic myometrial response are several orders of magnitude above those achieved in a clinical setting in the intact, live animal. It is possible,

therefore, that the synergistic negative inotropism is due to a toxic, histamine-like effect associated with the imidazole ring.

Another possible explanation for the left and downward shifts by idazoxan on the concentration response curve to xylazine might also be explained by membrane channel blockade, especially of voltage-sensitive sodium channels. It is reported that antagonist binding can lead to conformational changes in the receptor, not unlike that which occurs with agonist binding.⁵⁰ It has also been reported that the α_2 adrenoceptor antagonist, yohimbine, possess significant local anesthetic activity via voltage-sensitive sodium channel blockade.⁷⁸

It is also possible that the common action of xylazine and idazoxan is through alterations on ionic fluxes which would be accentuated in the presence of high concentrations ($\geq 10^{-4}$ M) of both compounds. Thus, the observed cumulative effects of a high concentration ($> 10^{-4}$ M) of idazoxan on the effect of xylazine on rat myometrium is, therefore, attributed to a blockade of voltage-sensitive calcium channels by xylazine, resulting in weaker smooth muscle contractions coupled with the blockade of voltage-sensitive sodium channels by both compounds. This would account for the dramatic reduction in contractile force of the smooth muscle when both compounds are present. The possibility of an action by one or both compounds at some unidentified receptor(s) still remains, however.

It is also of interest to speculate that α_2 adrenoceptors may not yet be present in sufficient numbers, or may be non-functional, in the virgin rat uterus. It, therefore, seems reasonable to suggest that the negative inotropic action of xylazine on isolated virgin rat uterine muscle is linked to either the local anaesthetic action of xylazine or a toxic effect associated with high concentrations of imidazoles. The relevance of these observations for the reproductive pharmacology and their relationship to the pathological states are important subjects for future investigations.

CHAPTER V

SUMMARY

From this study, the following conclusions can be made. Xylazine HCl produces a dose dependent negative inotropic effect on spontaneous contractions of virgin rat myometrium *in vitro* at concentrations greater than 1×10^{-4} M. Incubation of the tissues in high concentrations of idazoxan ($\geq 1 \times 10^{-4}$ M) for five minutes prior to and during xylazine exposure results in a leftward shift in the xylazine response curve indicating that idazoxan enhances xylazine's negative inotropic effect. A local anesthetic effect due to sodium channel blockade by xylazine and idazoxan most probably accounts for this response. However, additional actions by either compound at other membrane sites (ie, H₂-histamine receptors) cannot be ruled out.

CHAPTER VI

FUTURE INVESTIGATIONS

Further study is needed to answer the many questions raised by this study. Radioligand studies of virgin rat myometrium are necessary to confirm the presence of α_2 adrenoceptors and to determine the relative concentrations of α_1 to α_2 adrenoceptors in this tissue. The functionality of α_2 adrenoceptors needs to be assessed following chemical blockade of other receptor types present in the myometrium, in particular β and α_1 adrenoceptors. Knowledge of the stage of estrus at the time of tissue harvest would allow determination of the effect endogenous hormones on the α_2 adrenoceptor population of the virgin rat myometrium. This should be done in the naturally cycling female rather than in females treated with exogenous estrogen or progesterone.

Comparative studies among species, utilizing a standardized procedure, would allow us to determine if there truly are species differences in the sensitivity to xylazine. Comparing the sensitivity of ruminant, equine, and porcine tissues is of particular interest as these species display dramatic clinical differences in their sensitivity to xylazine (ruminants >> equine > porcine). Studies at the cellular level (cAMP production, ionic fluxes, etc) would shed light on the mechanisms by which xylazine and idazoxan express their physiological effects.

REFERENCES

1. Schummer A, Nickel R, Sack WO. *The Viscera of the Domestic Mammals*. 2nd revised ed. New York: Springer-Verlag, 1979:351-392.
2. Miller ME, Christensen GC, Evans HE. *Anatomy of the Dog*. Philadelphia: WB Saunders, 1964:779-791.
3. Pineda MH. Female reproductive system. In: McDonald, ed. *Veterinary Endocrinology and Reproduction*. 4th ed. Philadelphia: Lea & Febiger, 1989:303-354.
4. Roberts SJ. *Veterinary Obstetrics and Genital Diseases (Theriogenology)*. 3rd ed. Woodstock, VT: SJ Roberts, 1986:9.
5. Kawarabayashi T, Ikeda M, Sugimori H, Nakono H. Spontaneous electrical activity and effects of noradrenaline on pregnant human myometrium recorded by the single sucrose-gap method. *Acta Physiol Hung* 1986; 67:71-82.
6. Breuiller M, Rouot B, Litime M, Leroy M, Ferré F. Functional coupling of the α_2 -adrenergic receptor-adenylate cyclase complex in the pregnant human yometrium. *J Clin Endocrinol Metab* 1990;70:1229-1304.
7. Frid G, Hökfelt T, Lundberg JM, Terenius L, Hamberger L. Neuropeptide Y and noradrenaline in human uterus and myometrium during normal and pre-eclamptic pregnancy. *Hum Reprod* 1986;1:359-364.
8. Renegar RH, Rexroad CE. Uterine adrenergic and cholinesterase-positive nerves and myometrial catecholamine concentrations during pregnancy in sheep. *Acta Anat* 1990;137:373-381.
9. Kyozuka M, Crankshaw DJ, Crankshaw J, Berezin I, Kwan CY, Daniel EE. Alpha-2 adrenoceptors on nerves and muscles of rat uterus. *J Pharmacol Exp Ther* 1988;244: 1128-1138.
10. Guyton AC. *Textbook of Medical Physiology*. 5th ed. Philadelphia: WB Saunders, 1976:768-783.

11. Tietz WJ, Hall P. Autonomic nervous system. In: Swenson MJ, ed. *Duke's Physiology of Domestic Animals*. 9th ed. Ithaca, NY: Comstock Publishing, 1977:671-685.
12. Lumb WV, Jones EW. *Veterinary Anesthesia*. 2nd ed. Philadelphia: Lea & Febiger, 1984:43-73.
13. Tranquilli WJ, Greene SA. Cardiovascular medications and the autonomic nervous system. In: Short CE, ed. *Principles and Practice of Veterinary Anesthesia*. Baltimore: Williams & Wilkins, 1987:426-454.
14. Lefkowitz RJ, Hoffman BB, Taylor P. Neurohormonal transmission: The autonomic and somatic motor nervous systems. In: Gilman AG, Rall TW, Nies AS, Taylor P, eds. *Goodman and Gilman's The Pharmacological Basis of Therapeutics*. 8th ed. New York: Pergamon Press, 1990: 84-121.
15. Nichols AJ, Hieble JP, Ruffolo RR. The pharmacology of the peripheral α_1 - and α_2 -adrenoceptors. *Rev Clin Basic Pharm* 1988;7:129-205.
16. Taylor P, Brown JH. Acetylcholine. In: Siegel G, Agranoff B, Albers RW, Molinoff P, eds. *Basic Neurochemistry*. 4th ed. New York: Raven Press, 1989: 203-232.
17. Guyton AC. *Textbook of Medical Physiology*. 5th ed. Philadelphia: WB Saunders, 1976:608-625.
18. Erulkar SD. Chemically mediated synaptic transmission: An overview. In: Siegel G, Agranoff B, Albers RW, Molinoff P, eds. *Basic Neurochemistry*. 4th ed. New York: Raven Press, 1989:151-182.
19. Bylund DB. A historical perspective. In: Limbird LE, ed. *The alpha-2 Adrenergic Receptors*. Clifton, NJ: Humana Press, 1988:1-10.
20. Bylund DB. Heterogeneity of alpha-2 adrenergic receptors. *Pharmacol Biochem Behav* 1985;22:835-843.
21. Ahlquist RP. A study of the adrenotropic receptors. *Am J Physiol* 1948;153:586-606.

22. Delbarre B, Schmitt H. A further attempt to characterize sedative receptors activated by clonidine in chickens and mice. *Euro J Pharmacol* 1973;22:355-359.
23. Lands AM, Arnold A, McAuliff JP, Ludena FP, Brown TG. Differentiation of receptor systems activated by sympathomimetic amines. *Nature* 1967;214:597-598.
24. Wikberg JES. Pharmacological classification of adrenergic α receptors in the guinea pig. *Nature* 1978;273:164-166.
25. Berthelsen S, Pettinger WA. A functional basis for classification of α -adrenergic receptors. *Life Sci* 1977;21:595-606.
26. Bylund DB. Subtypes of α_2 -adrenoceptors: pharmacological and molecular biological evidence converge. *Trends Pharmacol Sci* 1988;9:356-361.
27. Langer SZ. Presynaptic regulation of the release of catecholamines. *Pharmacol Rev* 1981;32:337-357.
28. Weiner N, Molinoff PB. Catecholamines. In: Siegel G, Agranoff B, Albers RW, Molinoff P, eds. *Basic Neurochemistry*. 4th ed. New York: Raven Press, 1989: 233-252.
29. Borbujo J, Garcia-Villalón AL, Valle J, Gomez B, Dièquez G. Postjunctional *alpha*-1 and *alpha*-2 adrenoceptors in human skin arteries. An *in vitro* study. *J Pharmacol Exp Ther* 1989;249:284-287.
30. van Zwieten PA. Basic pharmacology of alpha-adrenoceptor antagonists and hybrid drugs. *J Hypertens* 1988;6(suppl 2):S3-S11.
31. Opie LH. *The Heart. Physiology and Metabolism*. 2nd ed. New York: Raven, 1991:195-207.
32. McGrath JC. Evidence for more than one type of postjunctional α -adrenoceptor. *Biochem Pharmacol* 1982; 31:467-484.
33. Langer SZ. Presynaptic receptors and their role in the regulation of transmitter release. *Br J Pharmacol* 1977;60:481-497.

34. Farnebo LO, Hamberger B. Drug induced changes in the release of [³H]-noradrenaline from field stimulated rat iris. Br J Pharmacol 1971;43:97-106.
35. Kirpekar SM, Puig M. Effect of flow-stop on noradrenaline release from normal spleens and spleens treated with cocaine, phentolamine and phenoxybenzamine. Br J Pharmacol 1971;43:359-369.
36. Langer SZ, Alder E, Engero A, Stefano FJE. The role of alpha receptors in regulating noradrenaline overflow by nerve stimulation. Proc Intl Congress Physiol Sci 1971;25:335.
37. Starke K. Influence of alpha-receptor stimulants on noradrenaline release. Natur Wissenschaften 1971;58: 420.
38. Arkinstall SJ, Moye I, Jones CT. α_2 -Adrenergic receptors in guinea pig myometrium in late pregnancy: Evidence for a predominantly postjunctional location. Am J Obstet Gynecol 1990;162:831-836.
39. Saarikoski S. Adrenoceptor function in female genital tract. Gynecol Obstet Invest 1988;26:56-62.
40. Hoffman BB, Lefkowitz RJ. Alpha-adrenergic receptor subtypes. N Eng J Med 1980;302:1390-1396.
41. Timmermans PB, Qian JQ, Ruffolo RR, van Zwieten PA. A study of the selectivity and potency of rauwolscine, RX 781094 and RS 21361 as antagonists of *alpha*-1 and *alpha*-2 adrenoceptors. J Pharmacol Exp Ther 1984;228: 739-748.
42. Reimann W, Scheider R. Presynaptic α_2 -adrenoceptors modulate the release of [³H]noradrenaline from rat spinal cord dorsal horn neurons. Eur J Pharmacol 1989; 167:161-166.
43. Daly RN, Sulpizio AC, Levitt B, DeMarinis RM, Regan JW, Ruffolo RR, Hieble JP. Evidence for heterogeneity between pre- and postjunctional *alpha*-2 adrenoceptors using 9-substituted 3-benzazepines. J Pharmacol Exp Ther 1988;247:122-128.

44. Wikberg JES. High affinity binding of idazoxan to a non-catecholaminergic binding site in the central nervous system: description of a putative idazoxan-receptor. *Pharmacol Toxicol* 1989;64:152-155.
45. Daly CJ, McGrath JC, Wilson VG. Evidence that the population of postjunctional-adrenoceptors mediating contraction of smooth muscle in the rabbit isolated ear vein is predominantly α_2 . *Br J Pharmacol* 1988;94: 1085-1090.
46. Vizi ES, Kapocsi J, Hársing GL, Del Tacca M. Heterogeneity of presynaptic alpha-2 adrenoceptors. *Acta Physiol Hung* 1990;75:297-298.
47. Haniuda M, Itoh M, Chiba S. Time-dependent enhancement of xylazine-induced, α_2 -adrenoceptor-mediated vasoconstriction in isolated and perfused canine pulmonary veins. *J Pharmacol Exp Ther* 1989;249:340-347.
48. MacDonald E, Ruskoaho H, Scheinin M, Virtanen R. Therapeutic applications of drugs acting on alpha-adrenoceptors. *Ann Clin Res* 1988;20:298-310.
49. Breuiller M, Rouot B, Leroy MJ, Blot P, Ferré F. Different α_2 -adrenoceptor densities in the circular and longitudinal layers of human near-term myometrium as revealed by [3 H]-idazoxan binding studies. *Gynecol Obstet Invest* 1988;26:14-20.
50. Regan JW. Biochemistry of the alpha-2 adrenergic receptors. In: Limbird LE, ed. *The alpha-2 Adrenergic Receptors*. Clifton, NJ: Humana Press, 1988:15-65.
51. Ruffolo RR, DeMarinis R, Wise M, Hieble JP. Structure-activity relationships for alpha-2 adrenergic receptor agonists and antagonists. In: Limbird LE, ed. *The alpha-2 Adrenergic Receptors*. Clifton, NJ: Humana Press, 1988:115-176.
52. Hieble JP, Sulpizio AC, Nichols AJ, Willette RN, Ruffolo RR. Pharmacologic characterization of SK&F 102078, a novel α_2 -adrenoceptor antagonist which discriminates between pre- and postjunctional α_2 -drenoceptors. *J Pharmacol Exp Ther* 1988;247:645-652.

53. Dabiré H. Idazoxan: A novel pharmacological tool for the study of α_2 -adrenoceptors. *J Pharmacol* 1986;17: 113-118.
54. Doxey JC, Roach AG, Smith CFC. Studies on RX 781094: a selective, potent and specific antagonist of α_2 -adrenoceptors. *Br J Pharmacol* 1983;78:489-505.
55. Michel MC, Brodde OE, Schnepel B, Behrendt J, Tschada R, Motulsky HG, Insel PA. [3 H]Idazoxan and some other α_2 -adrenergic drugs also bind with high affinity to a nonadrenergic site. *Mol Pharmacol* 1989;35:324-330.
56. Hoffman BB, Lavin TN, Lefkowitz RJ, Ruffolo RR. *Alpha* adrenergic receptor subtypes in rabbit uterus: Mediation of myometrial contraction and regulation by estrogens. *J Pharmacol Exp Ther* 1981;219:290-295.
57. Schmitt H, Fournadjiev G, Schmitt H. Central and peripheral effect of 2-(2,6-dimethylphenylamino)-4-H-5,6-dihydro-1,3-thiazin (Bayer 1470) on the sympathetic system. *Eur J Pharmacol* 1970;10:230-238.
58. Gerber JG, Nies AS. Antihypertensive agents and the drug therapy of hypertension. In: Gilman AG, Rall TW, Nies AS, Taylor P, eds. *Goodman and Gilman's The Pharmacological Basis of Therapeutics*. 8th ed. New York: Pergamon Press, 1990:784-813.
59. Greene SA, Thurmon JC. Xylazine--a review of its pharmacology and use in veterinary medicine. *J Vet Pharmacol Ther* 1988;11:295-313.
60. Gross ME, Tranquilli WJ. Use of α_2 -adrenergic receptor antagonists. *JAVMA* 1989;195:378-381.
61. Jansen CAM, Lowe KC, Nathanielsz PW. The effects of xylazine on uterine activity, fetal and maternal oxygenation, cardiovascular function, and fetal breathing. *Am J Obstet Gynecol* 1984;148:386-390.
62. Lumb WV, Jones EW. *Veterinary Anesthesia*. 2nd ed. Philadelphia: Lea & Febiger, 1984:187-190.
63. Gleed RD. Tranquilizers and sedatives. In: Short CE, ed. *Principles and Practice of Veterinary Anesthesia*. Baltimore: Williams & Wilkins, 1987:16-27.

64. Klude AM. Cardiopulmonary effects of xylazine in dogs. Am J Vet Res 1975;36:931-935.
65. Burns SJ, McMullen WC. Clinical application of Bay Vet 1470 in the horse. Vet Med 1972;67:77-79.
66. Ruffolo RR, Nichols AJ, Hieble JP. Functions mediated by alpha-2 adrenergic receptors. In: Limbird LE, ed. *The alpha-2 Adrenergic Receptors*. Clifton, NJ: Humana Press, 1988:187-253.
67. Knight AP. Xylazine. JAVMA 1980;176:454-455.
68. Doherty TJ, Ballinger JA, McDonell WN, Pascoe PJ, Valliant AE. Antagonism of xylazine induced sedation by idazoxan in calves. Can J Vet Res 1987;51:244-248.
69. Chiba S, Tsukada M. Pharmacological analysis of vasodilator responses to alpha₂-adrenoceptor agonists in isolated rat common carotid arteries. Jpn J Pharmacol 1990;53:135-143.
70. Marnet PG, Garcia-Villar R, Laurentie MP, Toutain PL. In vivo pharmacological characterization of alpha adrenergic receptors in sheep myometrium and their physiological meaning. Biol Reprod 1987;37:241-248.
71. LeBlanc MM, Hubbell JAE, Smith HC. The effect of xylazine hydrochloride on intrauterine pressure in the cow and the mare. Proceedings of Annual Meeting of the Society of Theriogenology 1984;211-220.
72. LeBlanc MM, Hubbell JAE, Smith HC. The effects of xylazine hydrochloride on intrauterine pressure in the cow. Theriogenology 1984;21:681-690.
73. Vass-Lopez A, Garcia-Villar R, Lafontan M, Toutain PL. [³H]Idazoxan binding to the ovine myometrium. Binding characteristic and changes due to steroid hormones. J Pharmacol Exp Ther 1990;253:819-824.

74. Rodriguez-Martinez H, McKenna D, Weston PG, Gustafsson BK, Whitmore HL. Uterine motility in the cow during the estrous cycle. III. Effects of oxytocin, xylazine, and adrenoceptor blockers. *Theriogenology* 1987;27:359-368.
75. Ko JCH, Evans LE, Hsu WH, Hopkins SM. Farrowing induction with cloprostetol-xylazine combination. *Theriogenology* 1989;31:795-800.
76. Ko JCH, Hsu WH, Evans LE. Xylazine-induced contractility in uterine strips obtained from cycling cows is mediated by α -2 adrenoceptors. 68th Annual Meeting of the Conference of Research Workers in Animal Disease, November 16 & 17, 1987. Abstract #137.
77. Minoia P, Mitolo-Chieppa D. Pharmacological and clinical properties of xylazine (in cow and rat). *Vet Bull* 1977;47:559.
78. Zimanyi I, Lajtha A, Vizi ES, Wang E, Reith MEA. Evidence that there is no direct correlation between alpha₂-adrenoceptor antagonism and inhibition of voltage-dependent sodium channels. *Neuropharmacology* 1988;27:1205-1208.
79. Sanger DJ. Behavioural effects of the alpha₂-adrenoceptor antagonists idazoxan and yohimbine in rats: comparisons with amphetamine. *Psychopharmacology* 1988;90:243-249.
80. Chapleo CB, Doxey JC, Myers PL, Roach AG. RX 781094, a new, potent, selective antagonist of α_2 -adrenoceptors. *Br J Pharmacol* 1981;174:842P.
81. Bourin M, Malinge M, Colombel MC, Larousse C. Influence of alpha stimulants and beta blockers on yohimbine toxicity. *Prog Neuro-psychopharmacol Biol Psychiatry* 1988;12:569-574.
82. Prud'Homme MJ. Effect of α -adrenergic blocking agents on uterine activity in the ewe at the end of gestation. *Acta Physiol Hung* 1988;71:485-489.
83. Rall TW. Oxytocin, prostaglandins, ergot alkaloids and other drugs; tocolytic agents. In: Gilman AG, Rall TW, Nies AS, Taylor P, eds. *Goodman and Gilman's The Pharmacological Basis of Therapeutics*. 8th ed. New York: Pergamon Press, 1990:933-952.

84. Haynes J, Pennefather JN. α_1 - and α_2 -Adrenoceptors of the circular myometrium from the dioestrous guinea-pig. *Clin Exp Pharmacol Physiol* 1988;15:695-701.
85. Wu YY, Goldfien A, Roberts JM. Alpha adrenergic stimulation reduces cyclic adenosine 3',5'-monophosphate generation in rabbit myometrium by two mechanism. *Biol Reprod* 1988;39:58-65.
86. Legrand C, Maltier JP, Benghan-Eyene Y. Rat myometrial adrenergic receptors in late pregnancy. *Biol Reprod* 1987;37:641-650.
87. Acritopoulou-Fourcroy S, Marçais-Collado H. Involvement of α -adrenoceptors in myometrial responses in the pro-oestral rat. *Br J Pharmacol* 1988; 93:185-191.
88. El Alj A, Breuiller M, Jolivet A, Ferré F, Germain G. α_2 -adrenoceptor response in the rat uterus at the end of gestation and after induction of labor with RU 486. *Can J Physiol Pharmacol* 1989;67:1051-1057.
89. Cruz A, Rudolph I. Adrenergic mechanisms in the control of myometrial activity in mice. Changes in estrous cycle. *Life Sci* 1986;38:2043-2051.
90. Berg G, Andersson RGG, Ryden G. α -Adrenergic receptors in human myometrium during pregnancy. *Am J Obstet Gynecol* 1986;154:601-606.
91. Buchanan GD, Garfield RE. Functional innervation of the myometrium of *Myotis lucifugus*, the little brown bat. *Biol Reprod* 1990;42:207-216.
92. Arkinstall SJ, Jones CT. [3 H]Rauwolscine binding to myometrial α_2 -adrenoceptors in pregnant guinea pig. *Am J Physiol* 1988;255:E272-E279.
93. Bottari SP, Vokaer A, Kaivez E, Lescrainier JP, Vauquelin GP. Differential regulation of α -adrenergic receptor subclasses by gonadal steroids in human myometrium. *J Clin Endocrinol Metab* 1983;57:937-941.
94. Arkinstall SJ, Jones CT. Myometrial α_1 -adrenoceptors in pregnant guinea pig: their distribution and increased number. *Am J Physiol* 1989;256:E215-E220.

95. Van Rossum JM. Cumulative dose-response curves II. Technique for the making of dose-response curves in isolated organs and the evaluation of drug parameters. *Arch Int Pharmacodyn* 1963;143:299-330.
96. Zar JH. *Biostatistical Analysis*. 2nd ed. Englewood Cliffs, NJ: Prentice Hall, Inc, 1984:43.
97. Hopkins TJ. The clinical pharmacology of xylazine in cattle. *Aust Vet J* 1972;48:109-112.
98. Riemer RK, Goldfien A, Roberts JM. Rabbit myometrial adrenergic sensitivity is increased by estrogen but is independent of changes in *alpha* adrenoceptor concentration. *J Pharmacol Exp Ther* 1987;240:44-50.
99. Legrand C, Maltier JP. Evidence for a noradrenergic transmission in the control of parturition in the rat. *Reprod Fert* 1986;76:415-424.
100. Insel PA, Motulsky HJ. Regulation of alpha-2 adrenergic receptors. In: Limbird LE, ed. *The alpha-2 Adrenergic Receptors*. Clifton, NJ: Humana Press, 1988: 281-322.
101. Cruz A, Sepúlveda WH, Rudolph I. Changes in the response to adrenergic drugs on mouse uterine contractions during pregnancy. *Life Sci* 1990;46:99-104.
102. Jedruch J, Gajewski Z, Ratajska-Michalczak K. Uterine motor responses to an α_2 -adrenergic agonist medetomidine hydrochloride in the bitches during the end of gestation and the post-partum period. *Acta Vet Scan* 1989;85:129-134.
103. Toro L, Stefani E, Erulkar S. Hormonal regulation of potassium currents in single myometrial cells. *Proc Natl Acad Sci USA* 1990;87:2892-2895.
104. Prud'Homme MJ. Uterine motor responses to an α -adrenergic agonist (phenylephrine) in the ewe during oestrus and at the end of gestation. *Reprod Nutr Develop* 1986;26:827-839.
105. Roberts JM, Insel PA, Goldfien A. Regulation of myometrial adrenoreceptors and adrenergic response by sex steroids. *Mole Pharmacol* 1981;20:52-58.

106. Fikes LW, Lin HC, Thrumon JC. A preliminary comparison of lidocaine and xylazine as epidural analgesics in ponies. *Vet Surg* 1989;18:85-86.
107. Stoelting RK. *Pharmacology and Physiology in Anesthetic Practice*. Philadelphia: JB Lippincott Co, 1987:156.
108. Mokhtari AR, Do Khac L, Harbon S. Forskolin alters sensitivity of the cAMP-generating system to stimulatory as well as to inhibitory agonists. A study with intact human platelets and guinea pig myometrium. *Eur J Biochem* 1988;176:131-137.
109. Convents A, DeBacker JP, Convents D, Vauquelin G. Tight agonist binding may prevent the correct interpretation of agonist competition binding curves for α_2 -adrenergic receptors. *Mole Pharmacol* 1987;32: 65-72.
110. Gierschik P, Jakobs KH. Mechanisms for inhibition of adenylate cyclase by alpha-2 adrenergic receptors. In: Limbird LE, ed. *The alpha-2 Adrenergic Receptors*. Clifton, NJ: Humana Press, 1988:75-114.
111. Isom LL, Limbird LE. What happens next? A hypothesis linking the biochemical and electrophysiological sequelae of alpha-2 adrenergic receptor occupancy with the diverse receptor-mediated physiological effects. In: Limbird LE, ed. *The alpha-2 Adrenergic Receptors*. Clifton, NJ: Humana Press, 1988:323-364.
112. Michel T, Hoffman BB, Lefkowitz RJ. Differential regulation of the α_2 -adrenergic receptor by Na^+ and guanine nucleotides. *Nature* 1980;288:709-711.
113. Stoelting RK. *Pharmacology and Physiology in Anesthetic Practice*. Philadelphia: JB Lippincott Co, 1987:800-801.
114. Bolton TB, Benham CD, Lang RJ, Takewaki T. Activity of single potassium channels recorded from single isolated visceral and vascular smooth muscle cells. In: Paton W, Mitchel J Turner P, ed. *IUPHAR 9th International Congress of Pharmacology, London 1984 Proceedings*. Vol 1. Basingstoke, Hampshire: Scientific & Medical Division, The Macmillan Press Ltd, 1984:173-177.

115. Wheaton LG, Rodriguez-Martinez H, Weston PG, Ko CH, Gustafsson BK. Recording uterine motility in the nonanesthetized bitch. Am J Vet Res 1986;10:2205-2207.
116. Rexroad CE, Barb CR. Contractile response of the uterus of the estrous ewe to adrenergic stimulation. Biol Reprod 1978;19:297-305.
117. Maigaard S, Forman A, Brogaard-Hansen KP, Anderson KE. Inhibitory effects of nitrendipine on myometrial and vascular smooth muscle in human pregnant uterus and placenta. Acta Pharmacol et Toxicol 1986;59:1-10.
118. Mironneau J, Savineau JP, Mironneau C. Effect of indapamide on the electromechanical properties of rat myometrium and rat portal vein. J Pharmacol Exp Ther 1986;236:519-525.
119. Limbird LE. Receptors linked to inhibition of adenylate cyclase: additional signaling mechanisms. FASEB J 1988;2:2686-2695.
120. Douglas, WW. Histamine and antihistamines: 5-hydroxytryptamine and antagonists. In: Goodman LS, Gilman A, eds. *The Pharmacological Basis of Therapeutics*. 5th ed. New York: MacMillan Publishing Co, 1975:590-629.

VITA

Meghan Richey was born September 27, 1957 in Baltimore, Maryland. After graduation with honors from Carroll County High School, Carrollton, Kentucky she attended Morehead State University, majoring in Pre-Veterinary Medicine. In 1977 she declared a major in Biology with a provisional teaching certificate but was accepted to Auburn University's School of Veterinary Medicine prior to completing the required course work for her Bachelor's degree.

She began her studies at Auburn's School of Veterinary Medicine in August, 1978 and was named to the Dean's list, joined the student chapters of the AVMA and AAEP, and was invited to join the Phi Zeta Society. For her senior seminar project she conducted original research in the evaluation of different collection sites on the results of trace mineral hair analysis in horses. After a preceptorship at a mixed animal practice in Ada, Oklahoma she received her DVM, with honors, in 1982.

Following graduation, Dr. Richey entered private practice in rural southwestern Pennsylvania. In January, 1984 she opened her own large animal ambulatory practice in Vanderbilt, Pennsylvania. In March of that year she also accepted a position in the Animal Health Technology program at Median School of Allied Health in Pittsburgh to teach large animal medicine and reproduction,

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In July, 1987, Dr. Richey began a residency program in veterinary anesthesia at Tufts University, School of Veterinary Medicine. She entered the Master's degree program at Virginia-Maryland Regional College of Veterinary Medicine, Virginia Polytechnic Institute and State University in January, 1989. The American College of Veterinary Anesthesiologists accepted her credentials for examination in 1990. She completed her residency at VMRCVM in June, 1991.