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Pharmacological characterization of the bovine median caudal artery

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Brent J. F. Hill

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DEDICATION

This thesis, submitted for the degree of master of science, is dedicated to my grandfather, Irving J. Fong, who was also known as "Pops." He was a strong believer in the personal development of people. A person can only develop as an individual if they possess the will and desire to do so. He wrote on a picture he once gave me, "you can do it so go get'em." "Pops" always felt that if a person is persistent and maintains a positive mental attitude, any goal is attainable.

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ABBREVIATIONS

NE	Norepinephrine
NO	Nitric Oxide
EDRF	Endothelium-derived relaxing factor
MAO	Monoamine oxidase
COMT	Catechol-O-methyltransferase
DOPA	3, 4-dihydroxyphenylalanine
PKC	Protein kinase C
IP_3	Inositol-1,4,5-triphosphate
DAG	Diacylglycerol
GDP	Guanosine diphosphate
GTP	Guanosine triphosphate
cAMP	Cyclic adenosine 3,5-monophosphate
ED_{50}	Effective agonist concentration required to elicit 50% of its maximal response
CR	The concentration ratio: $\frac{\text{agonist } EC_{50} \text{ in the presence of an inhibitor/antagonist}}{\text{agonist } EC_{50} \text{ in the absence of an inhibitor/antagonist}}$
pD_2	-log EC_{50}
K_B	Dissociation constant for an antagonist
pA_2	-log K_B
K_A	Dissociation constant for an agonist

CHAPTER 1. REVIEW OF LITERATURE

Anatomy of the bovine tail

The median caudal (coccygeal) artery starts at the first caudal vertebrate and continues down the length of the tail (Getty, 1975). This artery is an extension of the median sacral artery which originates from the abdominal aorta. The abdominal aorta is a segment from the descending aorta (Schaller, 1992; Getty, 1975). The median caudal artery is situated inside a vascular grove, which is enclosed by a pair V-shaped bones, called the hemoral processes, which originate from the caudal vertebrate (Getty, 1975; Dyce et al., 1987). It also runs directly ventral and parallel to the median caudal vein. Near the middle of each caudal vertebra, the median caudal artery branches out to form the ventral and dorsal branches (Getty, 1975).

The median caudal artery is also situated between the median ventral sacrocaudal muscles, these are the lateral flexor muscles of the tail (Evans and Christensen, 1979). These ventral median muscles have their highest muscle density at the second vertebra, but virtually disappear by the fourth vertebra. The limbs of animals are primarily comprised of slow-twitch muscle fibers. It is thought that "the increased occurrence of slow-twitch distally in the bovine tail indicates that the muscles are playing an increasing postural role at the expense of potential for rapid movement" (Young and Kenrick, p. 55, 1989).

Architecture of the median caudal artery

The bovine tail artery has yet to be histologically studied. Ashida et al. (1988) has studied the morphology of the bovine tail artery myocyte using electron microscopy, and this will be discussed later. In general, arteries are predominantly composed of three layers: the tunica intima, the tunica media, and the tunica adventitia. The tunica intima, a single layer of endothelial cells, lines the inner surface of the vessel lumen. The middle layer (tunica media) of the artery is separated from the tunica intima by a layer of connective tissue (lamina propria). The tunica media is composed of several layers of smooth muscle cells oriented circumferentially or longitudinally. These layers of smooth muscle cells vary in number depending on the size of the vessel. The outer arterial layer is absent of smooth muscle cells and is called the tunica adventitia. It is composed of collagen fibers that are wrapped around the tunica media layer. Axon varicosities innervate blood vessels along the tunica adventitia layer, and rarely penetrate into the tunica media layer (Hirst and Edwards, 1989). The density of adrenergic innervation and the neuromuscular junction width distance isn't homogeneous between tissues and along the length of the vessel (Furchgott, 1972; Nield and Zelcer, 1982; Merrillees et al., 1963). The neuromuscular junction width for blood vessels is usually between 50nM to 100nM (Nield and Zelcer, 1982).

Smooth muscle cells are characteristically spiral shaped, and have a length and diameter of approximately 40-60μm and 4μm, respectively (Hirst and Edwards, 1989). However, Ashida et al. (1988) found that the myocytes of the bovine tail artery were unique. They were spindle shaped and had a length greater than 125-150μm, and a diameter of 10-

15μm. Smooth muscle cells also possess a nucleus, mitochondria, and sarcoplasmic reticulum (Hirst and Edwards, 1989). The bovine tail artery myocytes contain very few mitochondria, which are located near the plasma membrane and nucleus (Ashida et al., 1988). The sarcoplasmic reticulum was also sparse, and was concentrated just under the plasma membrane. In fact, Ashida et al. (1988) found that the bovine tail artery myocyte has 60% less sarcoplasmic reticulum than the rat thoracic artery. Consequently, the sarcoplasmic reticulum has only a minor role in the regulation of the intracellular calcium concentration. The rise in the intracellular calcium concentration was primarily due to the influx of extracellular calcium, which Ashida et al. (1988) associated with gated calcium channels. Ashida and Blaustein (1987) also observed that when the intracellular calcium concentration exceeded the amount needed for a contraction, the efflux of excess calcium (80-90%) was mediated by the sodium-calcium exchanger (located in the plasma membrane), not the sarcolemma calcium pump. This contrasts with results from the rat aorta, where the ATP-driven calcium pump was found to be more important than the sodium-calcium exchanger (Ashida and Blaustein, 1987).

Mediators of vasoactivity

Contractility of arterial smooth muscle is predominantly regulated by the sympathetic nervous system, and by hormones synthesized and released into the bloodstream by the adrenal gland. The three major hormones synthesized by the medulla of the adrenal gland are norepinephrine (NE), epinephrine, and dopamine. A bioassay of the bovine adrenal medulla

indicated that epinephrine, NE, and dopamine are synthesized in the following amounts 4000, 1250, and 17 (expressed as μg/g tissue), respectively; these amounts constituted 76%, 23%, and 1% of the total (Holzbauer and Sharman, 1972). While the adrenal gland can release epinephrine, NE, and dopamine into the bloodstream, only NE is released by the prejunctional sympathetic neuron.

The biosynthesis of NE and epinephrine follow identical pathways, starting with the precursor, tyrosine. The rate-limiting enzyme, tyrosine hydroxylase, converts tyrosine to 3,4-dihydroxyphenylalanine (DOPA). DOPA is then converted to dopamine by dopa-decarboxylase. Dopamine is oxidized by dopamine-β-hydroxylase to NE. Phenylethanolamine-N-methyltransferase in the adrenal gland is responsible for the conversion of NE to epinephrine. Catecholamine synthesis, within the nerve terminal, primarily occurs in the varicosities of the prejunctional nerve terminal. However, the conversion of dopamine to NE occurs in conjunction with storage vesicles located in the nerve terminal. Norepinephrine is stored within these vesicles and quantally released when the nerve is stimulated (Katzung, 1992; Euler, 1972).

The vascular endothelium (tunica intima) is important in the regulation of smooth muscle tone. The endothelium has been reported to depress the contractility of blood vessels to catecholamines (Oriowo et al., 1987; Doggrell, 1992; MacLean et al., 1993; Kaneko and Sunano, 1993). This suggests that the endothelium has a vasorelaxant effect on vessels. Endothelium-derived relaxing factor (EDRF), now thought to be primarily nitric oxide (NO), is released from the vascular endothelium and mediates the vasodilation (Ignarro et al., 1987;

Palmer et al., 1987). Results have demonstrated that removal of the endothelium enhances the contractility of blood vessels to a similar magnitude as inhibiting the synthesis of NO (MacLean et al., 1993; Vo et al., 1992). Coincidentally, Palmer et al. (1987) suggested that NO and EDRF are identical compounds. Ignarro et al. (1988) later proved, in the bovine intrapulmonary artery, that NO was responsible for the vascular relaxation induced by EDRF. Nitric oxide mediates the vasorelaxant effect of endothelium-dependent vasodilators, like bradykinin, prostaglandin D_2, and acetylcholine (Ignarro et al., 1988; Fisher-Nakielski and Schror, 1990; Braun and Schrör, 1992; Ayajiki et al., 1993).

Nitric oxide is released spontaneously (basal release) by blood vessels, such as the bovine coronary artery (Purdy and Milburn, 1991). Vo et al.(1992) suggested that NO is released from endothelial cells to oppose endothelial shear stress, thereby limiting blood vessel damage during vasoconstriction. Endothelial shear stress is caused by an increase in vascular tone, thereby changing the blood flow velocity (Vo et al., 1992). Activation of α_1- and α_2-adrenoceptors may also mediate the synthesis and release of NO (Kaneko and Sunano, 1993; MacLean et al., 1993). The amount of NO released from the endothelium appears to be directly proportional to the magnitude of the smooth muscle contraction, and occurs without any change in the membrane potential of the endothelial cell (Vo et al., 1992; Komori et al., 1988). Bradykinin, a vasodepressor, also releases NO in a concentration-dependent manner (Palmer et al., 1987). These results indicate that NO contributes to the maintenance of vessel tone.

Catecholamine disposition mechanisms

Norepinephrine is predominantly stored in synaptic vesicles within the prejunctional nerve terminal. Following sympathetic nerve stimulation, NE is quantally exocytosed and released into the neuromuscular junction. The released NE is then able to bind to prejunctional α_2-adrenoceptors which inhibit the release of NE, or diffuse across the junctional cleft to react with postjunctional α-adrenoceptors (Hirst and Edwards, 1989). The concentration of catecholamines in the neuroeffector junction, whether originating from the sympathetic neuron or bloodstream, is controlled by several disposition mechanisms. Within the junctional cleft NE can either be inactivated by catechol-O-methyltransferase (COMT) found within the plasma, or diffuse out of the synapse into the circulation (Coquil et al., 1973; Trendelenburg, 1972). The remaining NE, within the neuroeffector junction, can be actively taken back into the prejunctional nerve terminal by uptake$_1$ (neuronal uptake), or be taken up by the effector tissue by uptake$_2$ (extraneuronal uptake). Upon uptake into the nerve terminal or effector cell, NE undergoes enzymatic inactivation by COMT and by monoamine oxidase (MAO) (Levin and Furchgott, 1970; Trendelenburg, 1972; Verity et al., 1972).

Uptake$_1$ is a saturable carrier-mediated transport system for catecholamines, and is located along the medial-adventitial border (site of adrenergic innervation) of the artery (Trendelenburg, 1990; Levin and Furchgott, 1970). Therefore, catecholamines circulating in the bloodstream need to diffuse across the tunica media layer of the artery to reach uptake$_1$ (de la Lande, 1989; Morris et al., 1988). Uptake$_1$ also acts by "facilitated exchange diffusion" to indirectly mediate the release of NE from the prejunctional nerve terminal

(Trendelenburg, p. 15, 1990). "Facilitated exchange diffusion," predominantly associated with indirectly acting sympathomimetic amines like tyramine and guanethidine, occurs when substrates for uptake$_1$ are also substrates for the vesicular uptake mechanism associated with storage vesicles located within the prejunctional nerve terminals (Trendelenburg, p. 15, 1990; Miyahara and Suzuki, 1985). These indirectly acting amines are taken up into the nerve terminals by uptake$_1$, and if not deaminated by MAO, act to displace NE from the storage vesicles (Miyahara and Suzuki, 1985; Furchgott et al., 1963).

Uptake$_2$ is a carrier-mediated transport system that favors the inward transport of positively charged protonated substrates (Schömig et al., 1992). It is primarily found within the tunica media smooth muscle layer of the artery (de la Lande, 1989; Morris et al., 1988). High concentrations of catecholamines are able to saturate this uptake mechanism (Schömig and Schönfeld, 1990). The membrane potential of the effector cell provides the driving force for uptake$_2$. The inside negativity of the cell facilitates uptake and hinders the efflux of substrates. Therefore, depolarization of the effector cell prevents substrate uptake and causes the outward efflux of substrates (Schömig et al., 1992). Pharmacological inhibitors for uptake$_2$ are able to impair both the influx and efflux of catecholamines within the effector cell (Eckert et al., 1976).

Catecholamines undergo deamination by MAO. Monoamine oxidase is a mitochondrial enzyme located in both the adrenergic nerve terminal and the effector cell (Coquil et al., 1973; Verity et al., 1972; Levin and Furchgott, 1970). Monoamine oxidase exists in two forms: type A and type B. Type A is found within sympathetic nerve terminals,

brain, and liver, while type B has been found in the arterial walls of rats and in the rabbit aorta (Coquil et al., 1973). Norepinephrine and serotonin are primarily metabolized by type A MAO (Rivett et al., 1982; Precious and Lyles, 1988). Type B metabolizes NE to a lesser degree than type A. Tyramine is a good substrate for both MAO types (Caramona, 1982; Precious and Lyles, 1988). Trendelenburg et al. (1987) found that the K_m for MAO in the nerve terminal is higher than the K_m for $uptake_1$. Therefore, MAO is not saturated by the activity of $uptake_1$.

Catechol-O-methyltransferase (COMT) is primarily found within the tunica media layer of blood vessels, and is responsible for the O-methylation of catecholamines (Verity et al., 1972; Levin and Furchgott, 1970). The activity of COMT is dependent on the influx of catecholamines through the $uptake_2$ mechanism (Martel et al., 1993; Graefe and Trendelenburg, 1974). This enzyme can be saturated at high concentrations of catecholamines since its K_m is lower than that of $uptake_2$ (Henseling, 1980). When COMT is saturated, the excess catecholamines in the effector tissue are transported back out of the tissue through $uptake_2$, thereby maintaining a steady-state balance between the uptake of catecholamines and the rate of COMT activity (Eckert et al., 1976). Within the effector cell, COMT is more important than MAO in catecholamine metabolism (Eckert et al., 1976; Henseling, 1980; Kalsner and Nickerson, 1969; Schömig et al., 1992). This is partially because COMT and MAO function is series. Since MAO is a mitochondrial enzyme and COMT is predominantly located in the cytosol, it can be hypothesized that once

catecholamines are taken up by uptake$_2$, they are first exposed to COMT before they can reach the mitochondria for inactivation (Kalsner and Nickerson, 1969).

The catecholamine concentration in contact with the various disposition mechanisms is determined by the thickness of the tissue, the width of the neuromuscular junction, and the density of adrenergic innervation (Trendelenburg, 1972). Catecholamines, circulating in the bloodstream, have a higher concentration within the lumen of the artery (intima layer) than at the adventitia layer (de la Lande, 1989). These catecholamines diffuse across the three layers of the artery (intima, media, and adventitia) as determined by these catecholamine concentration gradients found across the arterial wall. However, in large arteries, the vasovasorum and lymph may contribute to maintaining the catecholamine concentration in the adventitial layer similar to that in the lumen. The media layer has the lowest diffusion coefficient, therefore making it a major obstacle for amine diffusion. This suggests that the rate of catecholamine uptake, by uptake$_1$ or uptake$_2$, is linked to the ability of the amine to diffuse across the arterial layers (de la Lande, 1989). The concentration of catecholamines within the neuromuscular junction is inversely proportional to the distance between the nerve terminal and effector tissue (width of the neuromuscular junction). As the width of the neuroeffector junction increases, the neuronally released catecholamines have to traverse a larger distance to reach the effector tissue. Therefore, the potential for the amines to diffuse away from the synapse into the circulation increases. The activity of uptake$_1$ is dependent on the distance between the nerve terminal and the effector tissue. As the distance increases, the

importance of $uptake_1$ in disposing catecholamines decreases. This was demonstrated on the inferior and medial muscles of the cat nictitating membrane (Trendelenburg, 1972).

The density of adrenergic innervation (therefore $uptake_1$) also regulates the amount of NE exposed to the effector disposition mechanisms (Schömig et al., 1992; Morris et al., 1988; Burnstock et al., 1972; Gaefe and Trendelenburg, 1974). This was confirmed in the cat nictitating membrane (Graefe and Trendelenburg, 1974). Hydrocortisone, an $uptake_2$ inhibitor, failed to potentiate the catecholamine response in the cat nictitating membrane. However, after the nictitating membrane was denervated, hydrocortisone potentiated the NE response. This suggested that the high activity of $uptake_1$ in the nictitating membrane had significantly decreased the NE concentration within the neuroeffector junction. Therefore, the significance of $uptake_2$ in the inactivation of NE was not apparent until the tissue was denervated (Graefe and Trendelenburg, 1974). Wyse (1974), in his studies on rabbit aortic strips, estimated that 50% of exogenously administered NE and 90% of neuronally released NE was taken up by $uptake_1$. Since the rabbit aorta is not well innervated, it is hypothesized that the importance of $uptake_1$ increases in more highly innervated tissues, while the activity of $uptake_2$ decreases (Burnstock et al., 1972; Wyse, 1974; Graefe and Trendelenburg, 1974). Schömig et al. (1992) demonstrated that there was more accumulation of ^{3}[H]NE at the surface ($uptake_1$) of the more highly innervated rat vas deferens than in the rat atrium. Coincidentally, there was also a greater percentage of [^{3}H]NE in the center of the muscle ($uptake_2$) of the less densely innervated rat atrium than in the rat vas deferens. They

concluded that uptake$_2$ has only a minor role in the removal of [^{3}H]NE from the junctional cleft due to the high density of adrenergic innervation of the rat vas deferens.

The physiological role played by each catecholamine disposition mechanism has been ascertained using pharmacological inhibitors for the specific mechanism. The importance of each inhibited disposition mechanism is assessed by its ability to prolong and/or potentiate the response to catecholamines (Trendelenburg, 1972). Cocaine and corticosterone have been extensively used to block uptake$_1$ and uptake$_2$, respectively (Furchgott and Garcia, 1968; Furchgott et al., 1963; Wyse, 1974). The enzymatic mechanisms, MAO and COMT, have been studied using the inhibitors, iproniazid and tropolone, respectively (Furchgott and Garcia, 1968; Wyse, 1974).

Pharmacological differentiation of adrenoceptors

The catecholamines, NE and epinephrine, mediate their biological response by binding to receptors located on either the prejunctional or postjunctional cell membrane (Ruffolo, 1991). These receptors were classified by Ahlquist (1948) into two groups, α- and β-adrenoceptors, based on the relative potency of several agonists to initiate opposing responses (excitatory or inhibitory). Lands et al. (1967) further subdivided the β-adrenoceptors into β_1- and β_2-adrenoceptors. The α-adrenoceptors were also divided into two groups, α_1- and α_2-adrenoceptors, by Langer (1974). Adrenoceptors can be pharmacologically characterized by comparing the effective concentration of an agonist/antagonist to produce a response (relative potency of a drug). However, using

agonists to classify receptor types can be difficult since the response to the agonist is determined by the receptor's efficacy and affinity for the agonist. Therefore, receptors with a high affinity for the agonist, but having a low efficacy, may generate a low response or possibly no response at all (Drew, 1985). The predominant property governing the receptor's response to the antagonist, however, is affinity. Therefore, tissues having identical receptors for an agonist should yield relatively identical responses in the presence of a selective antagonist for the receptor (Drew, 1985; Arunlakshana and Schild, 1959). Receptors can also be purified and characterized by ligand-binding. This method also utilizes the affinity of a receptor for a particular antagonist (McGrath, 1983). Unfortunately, agonists and antagonists tend to lose their specificity for a specific adrenoceptor at high concentrations.

The agonists, NE and epinephrine, have approximately equal potencies at both α_1- and α_2-adrenoceptors, as does the antagonist, phentolamine. It is speculated, however, that α_2-adrenoceptors have a lower threshold for NE than α_1-adrenoceptors (McGrath, 1983; Drew and Whiting, 1979). Phenylephrine and cirazoline are selective α_1-agonists, while prazosin and WB4101 are selective α_1-antagonists (Wilson et al., 1991). The order of potency of some selective α_2-agonists are: medetomidine > clonidine = B-HT 920 > xylazine (Virtanen et al., 1988; Wilson et al., 1991). The selectivity of some common α_2-antagonists are idazoxan > rauwolscine > yohimbine (Wilson et al., 1991). Some selective α-adrenoceptor antagonists have a wide potency range, therefore, Medgett and Langer (1984) suggested that selective antagonists must be used over a 100-fold concentration range when characterizing receptors by Schild plot analysis. The selective α_1-adrenoceptor antagonist,

prazosin, has a selectivity for α_1-adrenoceptors, over α_2-adrenoceptors, that spans over a 100-fold concentration range. Coincidentally, the selective α_2-adrenoceptor antagonist, yohimbine, has a selectivity for α_2-adrenoceptors, over α_1-adrenoceptors, that also covers a 100-fold concentration range. α_1-Adrenoceptors are commonly classified as having a prazosin/yohimbine ratio of 100, while α_2-adrenoceptors have a yohimbine/prazosin ratio of 100 (Drew, 1985).

The potency order for β-adrenoceptors was defined by Furchgott (1972): isoproterenol > epinephrine > NE > phenylephrine. β_1-Adrenoceptors have an equal affinity for both epinephrine and NE, while β_2-adrenoceptors have a greater affinity for epinephrine than NE (Ruffolo, 1991). Isoproterenol has a greater affinity for β_2-adrenoceptors than β_1-adrenoceptors. Activation of β_2-adrenoceptors, by isoproterenol, also activates adenylyl cyclase to a greater degree than β_1-adrenoceptors (Green et al., 1992).

Characterization of α-adrenoceptors

Langer (1974) suggested that the α-adrenoceptors, as proposed by Ahlquist, were not a homogeneous population, and that they be divided into two subtypes (α_1 and α_2) based on their respective anatomical differences. He suggested that α_1-adrenoceptors are located postjunctionally, and α_2-adrenoceptors are located prejunctionally. Berthelsen and Pettinger (1977) later redefined Langer's definition based on a functional approach. They defined α_1-adrenoceptors as postjunctional receptors that mediate excitatory responses. The prejunctional receptors responsible for inhibiting the neuronal release of NE were then

classified as α_2-adrenoceptors (McGrath, 1983; Berthelsen and Pettinger, 1977). This functional classification was later disproved by Drew and Whiting (1979) who found postjunctional excitatory α_1- and α_2-adrenoceptors. These results suggest that α_1-adrenoceptors are located postjunctionally and mediate an excitatory response. Activation of α_2-adrenoceptors causes both an inhibitory and excitatory effect at the pre- and postjunctional cell membrane, respectively, in smooth muscle.

There does appear to be a difference in the vascular distribution of α_1- and α_2-adrenoceptors based on their relative distance from the nerve terminals. α_1-Adrenoceptors are primarily located along the adventitial-medial border, in close proximity to the sympathetic innervation of the artery. Therefore, released NE from the nerve terminals preferentially acts at α_1-adrenoceptors. α_2-Adrenoceptors are located farther away (extrajunctionally) from the nerve terminals near the intimal layer of the artery. It is hypothesized that α_2-adrenoceptors are predominantly stimulated by circulating epinephrine in the bloodstream (McGrath, 1983; Langer and Shepperson, 1982). This distribution of α-adrenoceptors has been demonstrated in the hind limb of the dog by Langer et al., (1980). However, Langer et al., (1980) also hypothesized that an additional α_1-adrenoceptor population resides extrajunctionally in conjunction with the population of α_2-adrenoceptors. Medgett and Langer (1986) using the rat tail artery, demonstrated that α_1- and α_2-adrenoceptors are both located extrajunctionally and in close proximity to the nerve terminals. These results suggest that α_2-adrenoceptors are distributed unevenly in blood

vessels, with their greatest density occurring near the vessel lumen (Medgett and Langer, 1986).

The distribution of α_1- and α_2-adrenoceptors is not homogeneous within tissues (Medgett and Langer, 1984; Atkinson et al., 1988). The rat vas deferens and rabbit urethra have a higher population of functional postjunctional α_2-adrenoceptors than α_1-adrenoceptors, while the reverse is true for the bovine oviductal arteries, rat anococcygeus muscle, and rat tail artery (Andersson et al., 1984; Drew and Whiting, 1979; Atkinson et al., 1988; Costa et al., 1992). Whether a homogeneous population of α-adrenoceptors exists in the peripheral vessels of the bovine is uncertain. Nevertheless, studies have indicated that NE has a high potency for α-adrenoceptors in the isolated bovine tail artery (Ashida et al., 1988) and isolated bovine dorsal pedal vein (Solomons et al., 1989). The isolated perfused bovine ear artery also elicits a significant pressor response when exposed to phenylephrine (Eghianruwa and Eyre, 1991).

There appears to be an inverse relationship in the α-adrenoceptor population along the length of the vessel. The density of α_1-adrenoceptors appears to predominate in the proximal part of the rat tail, while the density of α_2-adrenoceptors increases in the distal end (Medgett and Rajanayagam, 1984; Medgett, 1985; Rajanayagam and Medgett, 1987). There is some speculation that α_2-adrenoceptors are predominantly located in the smaller resistance vessels (Langer and Shepperson, 1982).

This lack of homogeneity for α-adrenoceptors makes identification of postjunctional α_2-adrenoceptors difficult (Medgett and Langer, 1984). Identification of α_2-adrenoceptors

has been conclusively demonstrated using pressor responses *in vivo* (Langer and Shepperson, 1982; Langer et al., 1980). However, identification of arterial α_2-adrenoceptors *in vitro* with selective agonists and antagonists has been controversial (Savino and Varela, 1991; Atkinson et al., 1988; Dunn et al., 1991). Dunn et al. (1991), using the isolated rabbit distal saphenous artery, suggested that a complex interaction occurs between postjunctional α_1- and α_2-adrenoceptors. Therefore, expression of α_2-adrenoceptors is dependent on the prior simulation of α_1-adrenoceptors. It has been speculated that increasing the tone of the vessel, similar to physiological conditions *in vivo*, helps to uncover postjunctional α_2-adrenoceptors. Angiotensin II and vasopressin have been used to potentiate the response of an α_2-agonist on the isolated rat tail artery (Templeton et al., 1989). However, angiotensin II did not always potentiate the response of an α_2-agonist on the isolated rat tail artery (Savino and Varela, 1991).

α_1- and α_2-Adrenoceptors utilize different second messenger pathways to mediate their contractile response. The contractions can either be phasic (response has a short duration) or tonic (response is prolonged and maintained) (Ford, 1995). α_1-Adrenoceptors are capable of mediating both phasic and tonic contractions. They mediate their effects by stimulating the phosphatidylinositol second messenger system. When activated, the α_1-adrenoceptor couples to a G-protein (G_p). This protein complex stimulates phospholipase C, thus catalyzing the hydrolysis of inositol-4,5-bisphosphate into inositol-1,4,5-triphosphate (IP_3) and diacylglycerol (DAG). IP_3 mediates the release of calcium from the sacroplasmic reticulum to induce a phasic contraction. DAG activates protein kinase C (PKC) to initiate a

tonic contraction. Once PKC is activated it phosphorylates proteins found along the actin filament of the smooth muscle cell, thus initiating a contraction. Protein kinase C also mediates the opening of potential-sensitive calcium channels to cause an influx of extracellular calcium (Wilson et al., 1992; Ford, 1995). This influx of extracelluar calcium is responsible for maintaining the tonic contractions. α_2-Adrenoceptors mediate their response by coupling to a G-protein (G_i). This protein complex inhibits adenylyl cyclase, thereby decreasing the cytosolic accumulation of cAMP. This results in an influx of extracelluar calcium through potential-sensitive channels to cause a prolonged tonic contraction (Ford, 1995; Ruffolo et al., 1991; Wilson et al., 1991). Ashida et al. (1988) demonstrated that 85% of the NE-induced contraction in the myocyte of the bovine tail artery was mediated by the influx of extracellular calcium, while only 15% was due to calcium mobilization from the sarcoplasmic reticulum. In the rat aorta, each mechanism was capable of generating 50% of the contraction induced by NE.

It has been well established that both α_1- and α_2-adrenoceptors, as originally proposed by Langer (1974), can be further subdivided into distinct subtypes. Investigators have proposed several classification schemes for the identification of α_1-adrenoceptor subtypes. The discrepancy between classification schemes and nomenclature is dependent on the techniques (functional contractions, radioligand binding, and molecular biology) and α_1-adrenoceptor antagonists used when differentiating between subtypes (Hieble et al., 1995). Recently (1995), the International Union of Pharmacology Subcommitte on Nomenclature for Adrenoceptors proposed that α_1-adrenoceptors can be clearly divided into the three

subtypes: α_{1A}, α_{1B}, and α_{1D}. A functional response, using selective α_1-adrenoceptor antagonists for each subtype, has been clearly attributed to each subtype. There are fewer uncertainties concerning the subclassification of α_2-adrenoceptors than the α_1-adrenoceptors. α_2-Adrenoceptors have been pharmacologically classified into four subtypes (α_{2A}, α_{2B}, α_{2C},and α_{2D}) using isolated cell lines and radioligand binding techniques (Bylund, 1992).

α_1-Adrenoceptors have been found to possess two binding sites that have either a high or low affinity for agonists/antagonists (Colucci et al., 1985; Morrow and Creese, 1986; Jagadeesh and Deth, 1987; Minneman, 1988). Epinephrine displacement studies for the [^{3}H]Prazosin binding site have revealed both high and low affinity binding sites in the bovine aorta from young animals (Jagadeesh and Deth, 1987; Jagadeesh et al., 1990). However, Jagadeesh et al. (1990) found that the high affinity α_1-adrenoceptor binding site disappears as an animal matures. Therefore, the α_1-adrenoceptors in the adult bovine were dominated by the low affinity binding site (Jagadeesh et al, 1990). The low affinity site comprised 77% of the α_1-binding sites on the bovine thoracic aorta from young animals (Jagadeesh and Deth, 1987). Jagadeesh and Deth (1987) proposed that these two binding sites utilize different second messenger pathways for initiating the biological response. When an agonist binds to an α_1-adrenoceptor, GDP is dislocated by GTP from the G-protein subunit. The high affinity site binds GDP tightly, and is inhibited by the binding of GTP to the G-protein subunit. However, the low affinity site does not bind GDP as tightly as the high affinity site, and is not affected by GTP-binding. Therefore when the GTP concentration is low, GDP is not highly displaced by GTP from the G-protein subunit of the high affinity site. As the

concentration of GTP increases within a cell, the high affinity site is capable of shifting to the low affinity site due to the additional binding of GTP to the G-protein subunit, thereby destabilizing the protein complex. This uncouples the α_1-adrenoceptor from the G-protein, therefore changing the second messenger cascade (Jagadeesh and Deth, 1987; Jagadeesh et al., 1990). Jagadeesh and Deth (1987), and Jagadeesh et al. (1990), proposed that the low affinity site is also less efficient than the high affinity site in mediating its biological response.

Characterization of β-adrenoceptors

Lands et al. (1967) refined the original classification of β-adrenoceptors, as proposed by Ahlquist, by suggesting the existence of two types of β-adrenoceptors based on functional differences. They defined β_1-adrenoceptors as those mediating lipolysis and myocardial contractility, while β_2-adrenoceptors were classified as causing bronchodilation and vasorelaxation. β_1-Adrenoceptors are located in the cardiac muscle, while β_2-adrenoceptors are predominantly located in blood vessels and other smooth muscle. However, the epicardial arteries of the heart are capable of possessing either subtype, depending on the animal species. The bovine descending coronary artery possesses a homogeneous population of β_1-adrenoceptors (Purdy and Stupecky, 1986). β_3-Adrenoceptors have been characterized in rat adipose tissue by Tan and Curtis-Prior (1983). They are also present in the canine cutaneous vasculature, and induce vasodilation (Berlan et al., 1994).

When activated by a ligand, β-adrenoceptors mediate their effects by coupling to the G-protein, G_s. This protein complex then stimulates adenylyl cyclase to increase the intracellular cAMP concentration (Green et al., 1992). Green et al., (1992) demonstrated that the β-subtypes have a difference in their coupling efficiency. At low epinephrine concentrations β_2-adrenoceptors activate adenylyl cyclase to a greater degree, and also have a greater coupling efficiency to the G_s protein, than β_1-adrenoceptors.

CHAPTER 2. STATEMENT OF THE PROBLEM

Little knowledge exists about the physiologic/pharmacologic factors which regulate the vasoactivity of blood vessels in the bovine. To remedy this lack of knowledge about the physiology/pharmacology of the bovine vasculature, we wanted to develop the isolated median caudal artery preparation as a model for the study of bovine blood vessels. The median caudal artery preparation is an attractive model to use since the bovine tail is readily available at slaughter plants. Previously, investigators have primarily used the following isolated vessel preparations in the study of the bovine vasculature: the coronary artery, the cerebral artery, the uterine artery, and the pulmonary artery and vein (Purdy and Stupecky, 1986; Foy et al., 1992; Ayajiki et al., 1993; Suzuki et al., 1984; Dyer, 1993; Ford et al., 1992; Cai et al., 1994; Ignarro et al., 1988) .

The purpose of this study was to determine the characteristics of this artery based on the following studies:

1.) To ascertain the importance of the catecholamine disposition mechanisms using inhibitors for uptake$_1$, uptake$_2$, monoamine oxidase (MAO), and catechol-O-methyltransferase (COMT).

2.) To ascertain the importance of the endothelium in regulating vasoactivity.

3.) To pharmacologically determine the functionality of α_1-, α_2-, and, β_2-adrenoceptors based on their affinity for selective agonists and antagonists.

4.) To determine the α-adrenoceptor receptor reserve and dissociation constant for NE.

CHAPTER 3. MATERIALS AND METHODS

Tissue preparation

Adult bovine tails were collected at a local abattoir and transported on ice back to the laboratory within 120 minutes after slaughter. The median caudal artery was dissected out of the proximal-medial part of the tail and cleared of extraneous connective tissue. The tissue was kept in a modified Krebs' solution with 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; and 0.03mM EDTA. Arteries were cut into ring segments 4mm in length. Through each lumen 2 triangular stainless steel wires were then inserted. The triangular wires were suspended by threads in a 10ml isolated tissue bath. One thread was looped under a stationary support near the bottom of the bath, while the other thread was connected to a Grass FT-03 force transducer. Tissue baths, containing Krebs' solution, were maintained at 37°C and aerated with a 95% O_2 - 5% CO_2 mixture. Tissue responses were recorded isometrically by a Grass or Beckman polygraph (model 7 or model R611, respectively). Ring segments were initially stretched to a tension of 10-12 grams (g) and then allowed to relax to a tension of 3g over a period of 60 minutes. Tissues were then equilibrated at a baseline tension of 2g for at least 30 minutes. Krebs' solution was replaced every 20 minutes unless otherwise indicated. For each experimental trial, adjacent rings from the same arterial segment were used.

Generation of concentration response-curves

In experiments to evaluate the effect of endothelium removal and L-NAME on the NE response and to determine the dissociation constant for NE and several antagonists, all arteries were treated with inhibitors for MAO, COMT, $uptake_1$, and $uptake_2$. During the 90 minute equilibration period all arteries were incubated with the MAO inhibitor, iproniazid (0.36mM), for 60 minutes and washed every 10 minutes over a 40 minute period. Before generating a concentration-response relationship to an agonist, the arteries were equilibrated with cocaine (3μM), corticosterone (10μM), and tropolone (10μM) for 20 minutes to block $uptake_1$, $uptake_2$, and COMT, respectively. If cocaine induced a contraction above baseline (2g tension), the experiment was discarded.

Administration of each agonist concentration was done cumulatively in approximately half-log increments. The response to each concentration was allowed to reach its maximum before the next concentration was added to the bath. The contractile response of each individual agonist concentration was then plotted. Data measurements were obtained at the effective agonist concentration required to elicit 50% of its maximal response (EC_{50}), as indicated by Schild (1949). In experiments that evaluated inhibitors for the catecholamine disposition mechanisms and the dissociation constants for NE and several α-adrenoceptor antagonists, a concentration-response curve was generated (and measured) in the absence (EC_{50}) and presence (EC_{50}*) of the inhibitor/antagonist under evaluation. The concentration ratio (CR) of the agonist EC_{50}*/EC_{50} was determined (Furchgott, 1972).

Adjusting for sensitivity changes

Any time-dependent shift of the agonist concentration-response relationship during the course of an experiment was monitored as recommended by Furchgott (1972), and as used in our laboratory (Zhang and Dyer, 1990). A "time control tissue," which was not treated with the drug under evaluation, was paired with each experiment. The concentration ratio (CR_T) for any time-dependent shift of the agonist concentration-response relationship (EC_{50} at time t /EC_{50} at time 0) was determined from the two control agonist concentration-response curves. The concentration ratio (CR) obtained for tissues treated with inhibitors and antagonists for the disposition mechanisms and adrenoceptors, respectively, were adjusted according to the formula: $CR=CR/CR_T$.

Evaluation of the norepinephrine disposition mechanisms

Factors regulating the NE concentration at the receptor site were evaluated by the ability of each enzymatic and uptake inhibitor to shift the concentration-response curve to the left. The disposition mechanisms for both fresh and cold stored (24 hrs.) tissues were evaluated.

Two enzymatic processes for catecholamine inactivation are monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT). Iproniazid (0.36mM) was used to inactivate MAO, and tropolone (10–100μM) was used to block COMT. To ascertain the importance of neuronal $uptake_1$, cocaine (1–100μM) and desipramine (0.3nM-10μM) were

used. Corticosterone 21-acetate (0.1–100μM), an extraneuronal $uptake_2$ inhibitor, was evaluated as well as its solvent, ethanol (10μl).

The equilibrated tissues were initially primed with 10μM NE for 10 minutes and then washed until they relaxed to baseline. Ring segments were then allowed to stabilize at baseline for 30 minutes. A control NE concentration-response relationship was generated and the tissues washed until they relaxed to baseline. Each concentration of corticosterone, ethanol, cocaine, desipramine, and tropolone was allowed to equilibrate with one ring of the artery segment for 30 minutes. Iproniazid was allowed to equilibrate with an arterial ring for 60 minutes, and then the tissue was washed every 10 minutes over a 40 minute period. This was followed by the determination of a second NE concentration-response relationship on all ring segments.

The data analysis for the ability of each inhibitor to shift the NE concentration-response curve to the left was evaluated at the EC_{50} of the NE concentration-response curve before (EC_{50}) and after (EC_{50}*) equilibration with the inhibitor. After correcting for sensitivity changes over time, a potentiation factor (PF) was calculated using the equation: $PF = (EC_{50})/(EC_{50}*)$.

The control NE concentration-response curves (0.01-10μM) were also analyzed based on the sex of the animals. Concentration-response curves to NE, for males and females, were analyzed at the EC_{50} and maximum response.

Evaluation of the response to tyramine

During equilibration, four ring segments were incubated with 0.36mM iproniazid for 60 minutes and then washed every 10 minutes over a 40 minute period. The tissues were then primed with 3μM of NE for 10 minutes, and washed until they relaxed to baseline. All the tissues were equilibrated with cocaine (3μM) for 20 minutes to block uptake$_1$. This was followed by the administration of 10μM NE to determine the control response. After the response to NE had reached a plateau, the tissues were washed (five times) for at least 30 minutes and allowed to stabilize at the baseline. Two of the four tissues were pretreated with 3μM cocaine for 20 minutes. This was followed by determining a NE and tyramine concentration-response relationship for both agonists in the presence and absence of cocaine. After determining the control response, the response to NE in the presence of cocaine was approximately similar to the control response. It was then assumed that after the tissues had been repeatedly washed following the determination of the control response, that cocaine did not remain in the tissue and alter the response of the tissue to the subsequent experimental procedures. The response produced by each respective agonist concentration administered in the absence and presence of cocaine was analyzed for significant differences.

The effect of endothelium removal on the norepinephrine response

One ring was denuded by the careful rotation of a blunt toothpick inside the lumen of the vessel, while the endothelium of the other ring was left intact. After the equilibration period, the two ring segments were primed with 3μM NE for 10 minutes and washed every

10 minutes for 40 minutes. This was followed by the administration of 120mM KCl. After obtaining a peak response to KCl, the tissues were washed and allowed to relax to baseline. The rings were pretreated with uptake and enzyme inhibitors, followed by the generation of a NE concentration-response relationship. The tissue's response to NE in the absence and presence of an endothelium was analyzed at their respective EC_{50} values.

The effect of L-NAME on the norepinephrine response

After the equilibration period, the two arterial ring segments were primed with 3μM NE for 10 minutes and washed every 10 minutes for 40 minutes. This was followed by the administration of 120mM KCl. After obtaining a peak response to KCl, the tissues were washed and allowed to relax to baseline. One tissue was pretreated with the nitric oxide synthase inhibitor, $^{\omega}$ N-nitro-L-arginine methyl ester HCl (L-NAME; 100μM), for 30 minutes. All rings were pretreated with uptake and enzyme inhibitors, followed by the generation of a NE concentration-response relationship. The NE concentration-response curves were analyzed at their EC_{50} in the absence and presence of L-NAME.

The effect of L-NAME on acetylcholine-mediated relaxation

After the equilibration period, the two ring segments were primed with 3μM NE for 10 minutes, and washed every 10 minutes for 40 minutes. One tissue was pretreated with the nitric oxide synthase inhibitor, L-NAME (100μM), for 30 minutes. Both tissues were initially contracted with 45mM KCl and allowed to stabilize at their maximum contractile

response. A cumulative acetylcholine concentration-response relationship (0.01 -100μM) was superimposed over the KCl-induced contraction. The response produced by each incremental increase in concentration of acetylcholine, in the absence and presence of L-NAME, was analyzed for significant differences.

Comparison of the contractile response to several agonists

Contractile responses to epinephrine, angiotensin II, 5-hydroxytryptamine (5-HT), medetomidine, B-HT 920, phenylephrine, and NE were evaluated using seven rings sectioned from the same artery segment. After equilibration, all the tissues were primed with 3μM NE for 10 minutes. The tissues were then washed and allowed to stabilize for 30 minutes at baseline. Tissues were then contracted with 120mM KCl. Following washout and relaxation to baseline, they were allowed to stabilize for 30 minutes before generating a cumulative concentration-response relationship to each agonist (0.1nM-100μM).

To maximize the agonist concentration at the receptor site tissues were pretreated, in the following way, with uptake and enzyme inhibitors. Tissue preparations for B-HT 920, epinephrine, phenylephrine, and NE were incubated with 0.36mM iproniazid for 60 minutes and then washed for 40 minutes. These four rings were pretreated with 1μM propranolol for 30 minutes to block β-adrenoceptors, and also pretreated with cocaine (3μM) and corticosterone (10μM) for 20 minutes to block $uptake_1$ and $uptake_2$, respectively. Tissues for 5-HT (serotonin) were pretreated with 3μM cocaine for 20 minutes before generating its concentration-response relationship. Tissues receiving medetomidine and angiotensin were

not equilibrated with uptake and enzyme inhibitors. The agonist concentration-response curves were analyzed at their EC_{50} and maximum response.

Evaluation of the response to isoproterenol

Two tissue rings were sectioned from the same artery segment. After equilibration, the tissues were pretreated with the α-adrenoceptor antagonist, phentolamine (1μM), for 45 minutes. Tropolone (10μM) and corticosterone (10μM) were added to the preparation for 20 minutes to block COMT and $uptake_2$, respectively. Both tissues were then contracted with 45mM KCl and the response was allowed to stabilize. Cumulative additions of isoproterenol (0.1-100μM) were superimposed upon the KCl-induced contraction of one tissue preparation. The second tissue served as a control in order to monitor relaxation with time.

The amount of relaxation, in both the time control and isoproterenol tissue preparations, was measured at the same point in time after each response to isoproterenol had stabilized. This relaxation amount was expressed as a percent of the maximum response to 45mM KCl in each tissue. The percent relaxation due to time was subtracted from the percent relaxation due to isoproterenol, thus obtaining the percent relaxation due to isoproterenol alone.

The effect of propranolol on the response to isoproterenol

During equilibration, the ring segments were incubated with the MAO inhibitor, 0.36mM iproniazid, for 60 minutes and washed every 10 minutes for 40 minutes. Tissues

were then primed with 3μM NE for 10 minutes and washed until they stabilized at baseline. One tissue served as a control and received no antagonist. The other tissues were equilibrated with one concentration of propranolol (30nM, 100nM, 1μM) for 60 minutes. All the tissues were pretreated with the nonselective α-adrenoceptor antagonist, phentolamine (1μM), for 60 minutes, and also pretreated with corticosterone (10μM) and tropolone (10μM) for 20 minutes to block uptake$_2$ and COMT, respectively. All the tissues were then contracted with 45mM KCl, and the responses were allowed to stabilize. An isoproterenol concentration-response relationship (0.01-100μM) was superimposed over the KCl-induced contraction. After the maximum response to 100μM isoproterenol was obtained, 10μM sodium nitroprusside was added to the tissues in order to ascertain if the tissues were capable of further relaxation.

Determination of the dissociation constant (K_A) for norepinephrine

After the equilibration period, ring segments were primed with 3μM NE for 10 minutes and washed every 10 minutes for 40 minutes. Before generating a NE concentration-response relationship, the rings were pretreated with uptake and enzyme inhibitors, and also propranolol (0.3μM) for 60 minutes to block β-adrenoceptors. A cumulative concentration-response relationship was initially generated to NE. Following washout and stabilization at the baseline, dibenamine (0.3μM for 20 minutes), an irreversible α-antagonist, was then added to one of the tissue baths to inactivate a fraction of the α-adrenoceptors. The other tissue served as a "time control tissue." After the dibenamine

treatment, the tissues were washed 4-5 times over a period of 30 minutes. This was followed by determining a second NE concentration-response relationship.

The methods and statistical analysis for determining the K_A of NE follow those as described by Furchgott and Bursztyn (1967). After correcting for time-dependent changes in sensitivity, the NE concentration-response curves before (1/[A]) and after (1/[A']) dibenamine treatment were plotted. A double reciprocal plot of equi-effective concentrations of NE before (1/[A]) and after (1/[A']) dibenamine treatment was made. This plot yields a slope of 1/q and an intercept of $(1-q)/q(K_A)$. Using the slope and abscissa intercept, the K_A and fraction of uninhibited receptors remaining, q, were calculated using the equation: $1/[A] = 1-q/q[A'] + 1/q(K_A)$. In this equation the fraction of receptors remaining after dibenamine treatment, q, is equal to the reciprocal of the slope. In simplifying this equation, K_A = slope-1/intercept. To estimate the receptor reserve the following equation was used (Ruffolo, 1982): K_A / EC_{50}.

Determination of dissociation constants (K_B) for several antagonists

Furchgott's (1972) methods were used to determine pA_2 and K_B values for the adrenoceptor antagonists, prazosin, phentolamine, rauwolscine, and idazoxan. The pA_2 for prazosin was determined against the selective α_1-agonist, phenylephrine, and the nonselective α-adrenoceptor agonist, NE. The pA_2 for phentolamine and idazoxan were determined against NE and phenylephrine, respectively.

After the equilibration period, the ring segments were primed with 3μM NE for 10 minutes and washed every 10 minutes for 40 minutes. Before determining agonist concentration-response relationships, the rings were pretreated with uptake and enzyme inhibitors, and also propranolol (0.3μM) for 60 minutes to block β-adrenoceptors. A control agonist concentration-response relationship was initially attained, and the tissues were washed until they stabilized at baseline. One concentration of either prazosin (3nM, 10nM, 30nM, 0.1μM), phentolamine (30nM, 0.1μM, 0.3μM,1μM), or idazoxan (3nM, 10nM, 30nM, 0.1μM, 0.3μM, 1μM), was then allowed to equilibrate for 60 minutes with each tissue before repeating the agonist concentration-response relationship.

K_B and pA_2 values were also determined for idazoxan, rauwolscine, and prazosin against the selective α_2-adrenoceptor agonist, medetomidine. After the equilibration period, ring segments were primed with 3μM NE for 10 minutes and washed every 10 minutes for 40 minutes. A concentration-response relationship to medetomidine was not generated before and after the tissue had equilibrated with the inhibitor (as was previously described using NE and phenylephrine) since medetomidine could not be washed out of the tissue after the determination of the control concentration-response relationship. Therefore, a control cumulative concentration-response relationship was determined to KCl (3-120mM) on all the tissues. The tissues were washed until they relaxed back to baseline. One tissue served as the control and received no antagonist. The other tissues were each equilibrated with one concentration of prazosin (3nM, 10nM, 30nM, 0.1μM, 0.3μM), rauwolscine (3nM, 30nM, 0.1μM), or idazoxan (10nM, 30nM, 0.1μM, 0.3μM, 1μM) for 60 minutes. All the tissues

were pretreated with uptake and enzyme inhibitors, and also propranolol (0.3μM) for 60 minutes to block β-adrenoceptors. This was followed by determining a medetomidine (10nM-100μM) concentration-response relationship. The response of each tissue to medetomidine was expressed as a percentage of its maximum response to KCl, and an EC_{50} value was determined. Unfortunately, prazosin depressed the response to medetomidine below the EC_{50} value for the response to KCl. Therefore, the response to medetomidine was expressed as grams tension developed, not as a percentage of the KCl response.

The pA_2 and K_B values were calculated according to Arunlakshana and Schild (1959), as described by Furchgott (1972), using the equation: $\log (DR-1) = \log [B] - \log K_B$. The contractile response of each individual agonist concentration in the absence and presence of an antagonist was plotted to obtain the effective agonist concentration required to elicit 50% of its maximal response (EC_{50}) as indicated by Schild (1949). As was previously mentioned, the concentration ratio (CR) was calculated at the EC_{50} of the agonist concentration-response relationship in the absence (A) and presence (A*) of each competitive antagonist concentration; whereby, CR = A*/A. After correcting for time-dependent changes in sensitivity, a Schild plot (Furchgott, 1972) of the log (CR-1) against the -log of each antagonist concentration, [B], was made to obtain the pA_2 value (abscissa intercept). If the blockage is competitive, the slope of the regression line should be unity. Under these dynamic conditions the $pA_2 = -\log K_B$.

The effect of tone on the tissue's response to medetomidine

Three tissues were equilibrated for 90 minutes and then primed with 3μM NE for 10 minutes. The tissues were washed (≥30 minutes) until they stabilized at baseline. 120mM of KCl was administered and the response was allowed to reach its maximum before washing out. After the baseline had stabilized at 2g, NE (0.3μM) and phenylephrine (0.3μM -3μM) were added to one of the two tissues, respectively, to induce a small contractile response just above threshold. The third tissue served as a control and received no agonist to induce tone. When the contractions had stabilized, a medetomidine cumulative concentration-response relationship was generated on all three tissues.

Statistical analysis

All results presented were corrected for sensitivity changes over time (see "Adjusting for sensitivity changes"). Results for each measurement are presented as mean ± S.E. The number of different animals, n, for each experiment is indicated. Agonist EC_{50} values were calculated using least squares linear regression on the steepest part of the concentration-response relationship. Significant differences between two means were tested using the two-tailed, paired or unpaired t-test (Eckblad, 1988; Netter et al., 1990). To test for significant differences between means and variances when there were more than two groups, the one-way analysis of variance (ANOVA) and F-max test were used, respectively (Eckblad, 1988; Netter et al., 1990). If the data was not normally distributed or did not have an equality of variance, then a power transformation was used on the data before using ANOVA. When a

significant difference was detected using ANOVA, the *a posteriori* sum of squares simultaneous test procedure was used to make multiple comparisons between the different means (Eckblad, 1988). In the Schild plot, the t-test (null hypothesis is that slope = unity) was used to determine if the slope was significantly different from unity (Eckblad, 1988). Differences were taken as significant when $p<0.05$.

Drugs

The following drugs were used: angiotensin II, (-)-epinephrine, (-)-isoproterenol, (-)-norepinephrine, cocaine, corticosterone 21-acetate, iproniazid, desipramine HCl, 5-hydroxytryptamine, sodium nitroprusside, ,$^{\omega}$ N-nitro-L-arginine methyl ester HCl (Sigma Chemical Co., St. Louis, MO); dibenamine HCl (Smith, Kline, and French, Philadelphia, PA); medetomidine HCl (Farmos Group Ltd., Turku, Finland); prazosin HCl (Pfizer Inc., Brooklyn, NY); propranolol HCl (Ayerst Laboratories, Inc., New York, NY); tropolone (Aldrich Chemical Co., Milwaukee, WI); tyramine (Calbiochem., La Jolla, CA); phentolamine mesylate and rauwolscine HCl (Research Biochemicals Inc., Natick, MA); phenylephrine HCl (Winthrop Laboratories, New York, NY); idazoxan (Kingston-Upon-Hull, U.K.); B-HT 920 (Boehringer, Indelheim, Germany). All drugs were dissolve in 9% saline except for corticosterone 21-acetate and dibenamine which were dissolved in ethanol.

CHAPTER 4. RESULTS

Methodological considerations

Several laboratories, including our laboratory (Zhang and Dyer, 1990), have stored fresh tissue overnight in a cold room to be reused the following day (Shibata et al., 1971; Langer et al., 1974). In our preliminary studies of the catecholamine disposition mechanisms, it was observed that tissue sensitivity significantly increased when tissues were cold stored (4°C) for twenty-four hours (n=5). The maximum contractile response produced by 30μM NE in fresh and cold stored tissues was 6.40 ± 0.62g and 9.51 ± 0.64g , respectively ($p<0.02$). The response to NE in the presence of the COMT inhibitor, tropolone (10μM; n=3), or the uptake$_1$ inhibitor, cocaine (1-100μM; n=3), differed between fresh and cold stored tissues. These inhibitors did not significantly potentiate the NE response in cold stored tissues. Since fresh and cold stored tissues differed in their response to NE, only fresh tissues were used in subsequent experiments. This was similarly done by Nair and Dyer (1974) using guinea-pig umbilical vessels. They found that the contractility of the umbilical vessels to serotonin, cold stored (4°C) for more than four hours, differed from fresh tissue.

The concentration-response curves to NE showed no significant difference in potency and maximum response between the male and female median caudal arteries (n=6). The EC_{50} of male and female arteries was 5.28 ± 0.61μM and 7.51 ± 1.77μM, respectively. The maximum response to 10μM NE on the median caudal artery from male and female bovine

was 6.92 ± 1.01g and 7.86 ± 1.31g, respectively. Since there was not a significant difference between male and female arteries in their response to NE, the arteries were not separated based on sex in subsequent experiments.

Evaluation of the disposition mechanisms for norepinephrine

The sensitivity of the tissue to NE is partially determined by the NE concentration within the neuromuscular junction and at the receptor site. A reduction in the NE concentration within the junctional cleft by the enzymatic (MAO and COMT) and uptake mechanisms ($uptake_1$ and $uptake_2$) may reduce the response of the tissue to NE. Inhibition of each of these disposition mechanisms should increase the response of the tissue to NE if that mechanism has an important role in the inactivation of NE within the neuromuscular junction.

The enzymatic inactivation of norepinephrine was studied using iproniazid and tropolone, which are inhibitors for monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT), respectively. Tropolone (10μM; n=5) significantly ($p<0.01$) shifted the EC_{50} of the NE concentration-response curve to the left approximately 1.7-fold. However, there was no significant potentiation of the response to NE using 100μM tropolone (n=5; Figure 4.1). No potentiation of the NE response was ascertained using 0.36mM iproniazid (n=6; Figure 4.2).

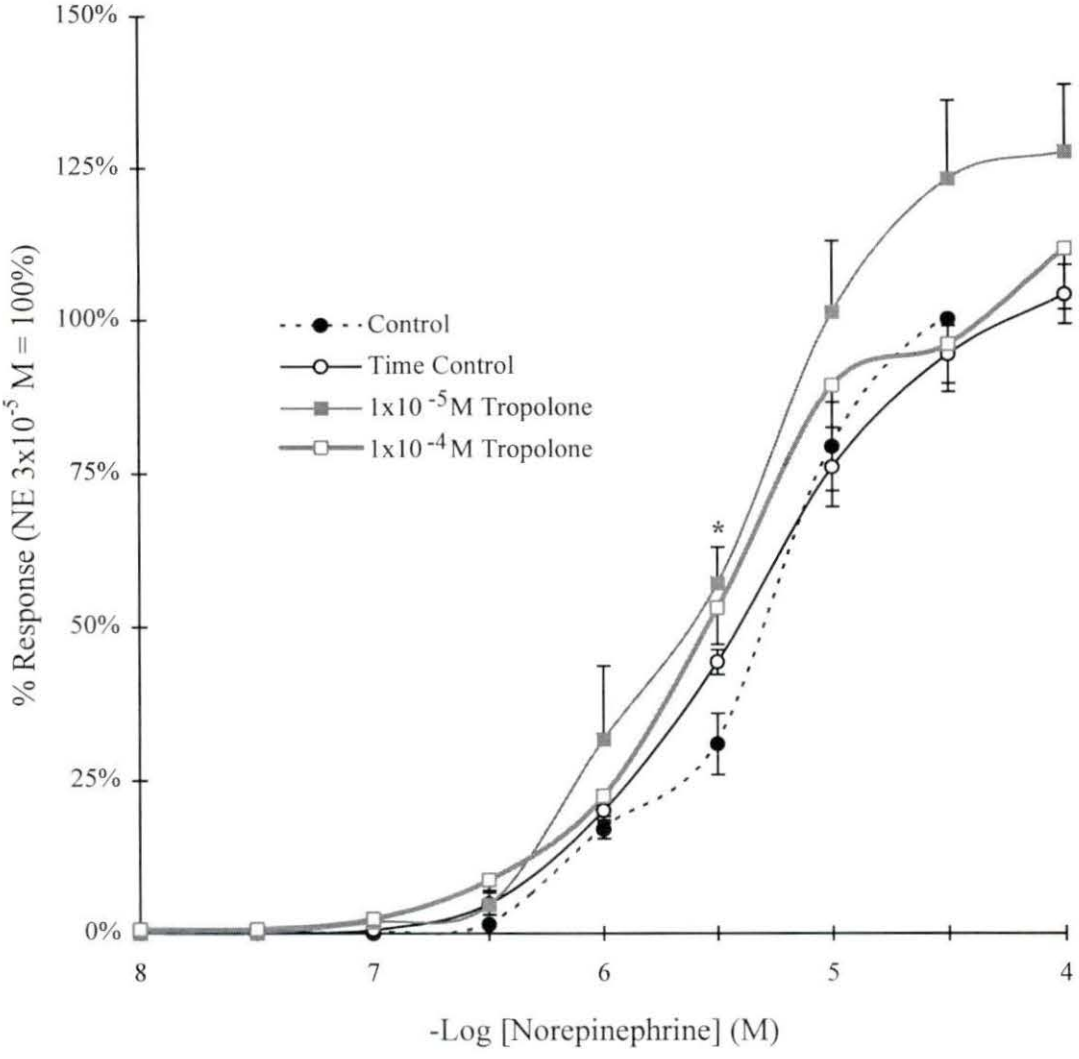

Figure 4.1. The effect of tropolone pretreatment (30 minutes) on the contractile response to norepinephrine. An asterisk (*) indicates points on the curve ($1x10^{-5}$ M tropolone) which are significantly different from the time control response. Each data point represents the mean ± S.E. for 5 animals.

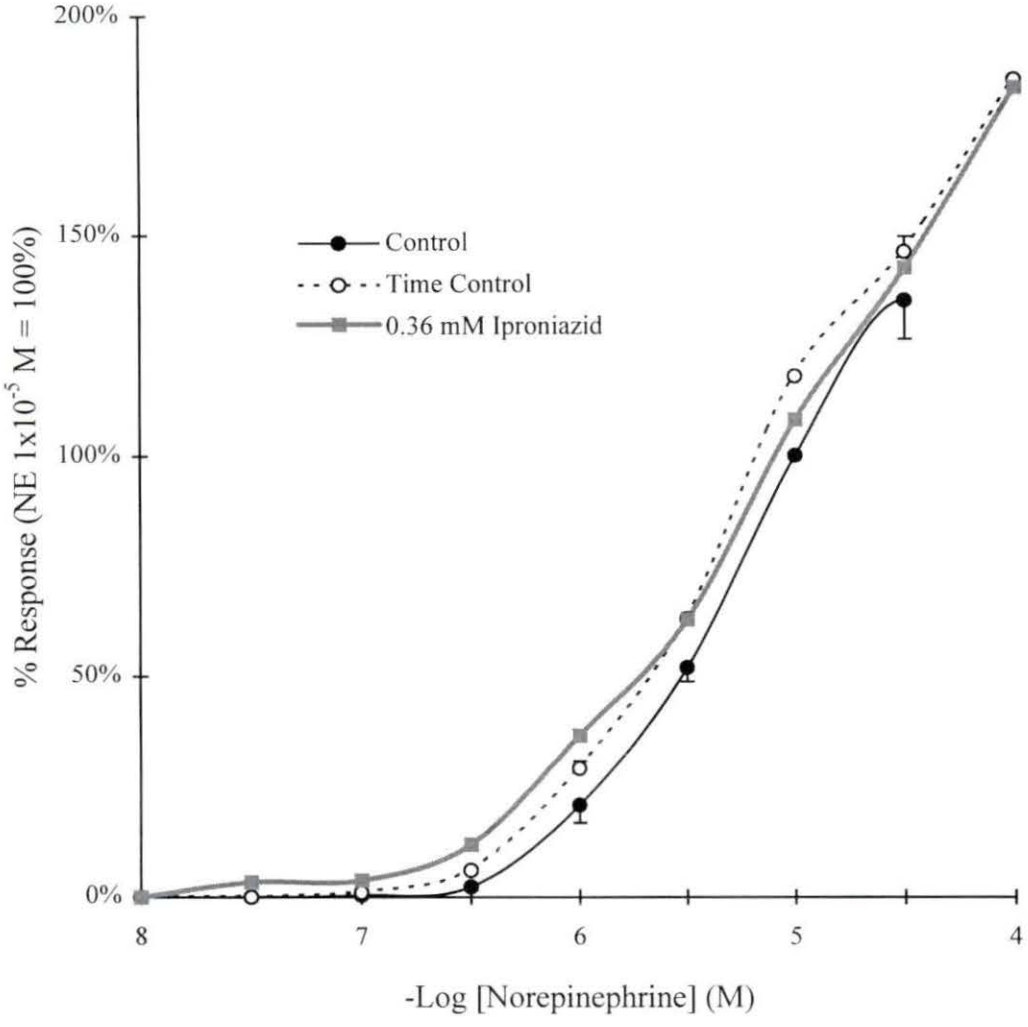

Figure 4.2. The effect of iproniazid pretreatment (60 minutes) on the contractile response to norepinephrine. Each data point represents the mean ± S.E. for 6 animals.

Extraneuronal uptake$_2$ was evaluated using corticosterone 21-acetate (n=5). The NE concentration-response curve was shifted three-fold to the left using corticosterone concentrations of 1-10μM ($p<0.02$; Figure 4.3). The solvent for corticosterone, 10μl ethanol, did not significantly affect the EC_{50} for NE (n=5; Figure 4.4).

Neuronal uptake$_1$ was evaluated using the inhibitors, desipramine (n=5) and cocaine (n=6). Desipramine (0.3nM-10μM) failed to potentiate the response to NE. However, there was significant inhibition ($p<0.05$) of the response to NE by 10μM desipramine, which shifted the NE EC_{50} to the right approximately fifteen-fold (Figure 4.5). Unlike desipramine, cocaine significantly shifted the NE concentration-response curve to the left three-fold at concentrations of 10μM ($p<0.001$) and 100μM ($p<0.01$), however the potentiation by 1μM cocaine was not significant (Figure 4.6). Occasionally, cocaine at concentrations from 3-100μM induced contractions. These experiments were excluded from the study. Since 10μM cocaine induced contractions more frequently than 3μM cocaine, 3μM cocaine was used to block uptake$_1$ in subsequent experiments.

The indirect agonist, tyramine, was used to investigate the importance of the uptake$_1$ disposition mechanism. Tyramine acts through uptake$_1$ to release NE from storage vesicles found within the prejunctional nerve terminal. Therefore, a tyramine-induced contraction indicates that stored NE is present in the nerve terminal. Blocking uptake$_1$ with cocaine should then decrease the amount of NE released by tyramine within the prejunctional nerve terminal (Furchgott, 1963). Cocaine (3μM) depressed the response to tyramine, but the magnitude of the effect was not significant (n=4; Figure 4.7). Cocaine (3μM) was also found

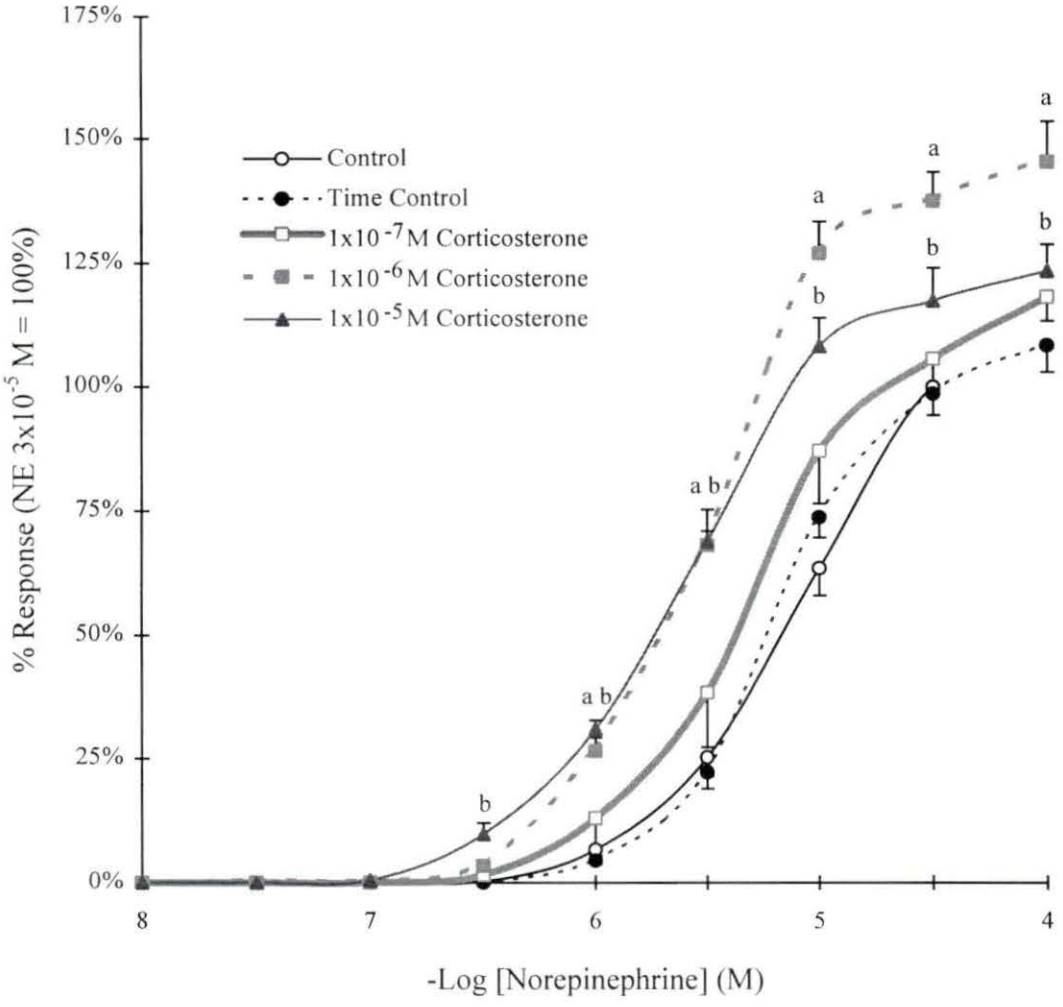

Figure 4.3. The effect of corticosterone pretreatment (30 minutes) on the contractile response to norepinephrine. Letters (a = $1x10^{-6}$ M corticosterone, b = $1x10^{-5}$ M corticosterone) indicate points on the curves that are significantly different from the time control response. Each point represents the mean ± S.E. for 5 animals.

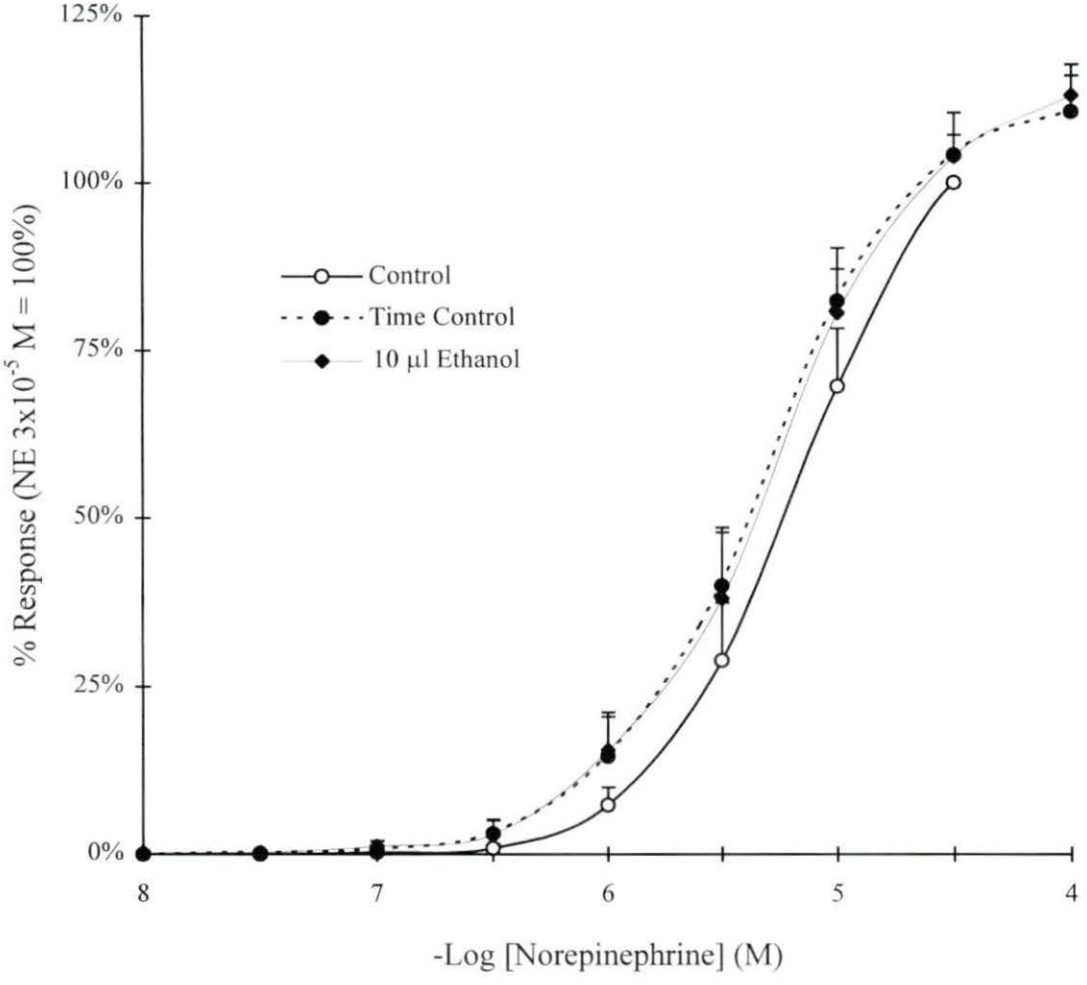

Figure 4.4. The effect of ethanol (10µl) pretreatment (30 minutes) on the contractile response to norepinephrine. Each data point represents the mean ± S.E. for 5 animals.

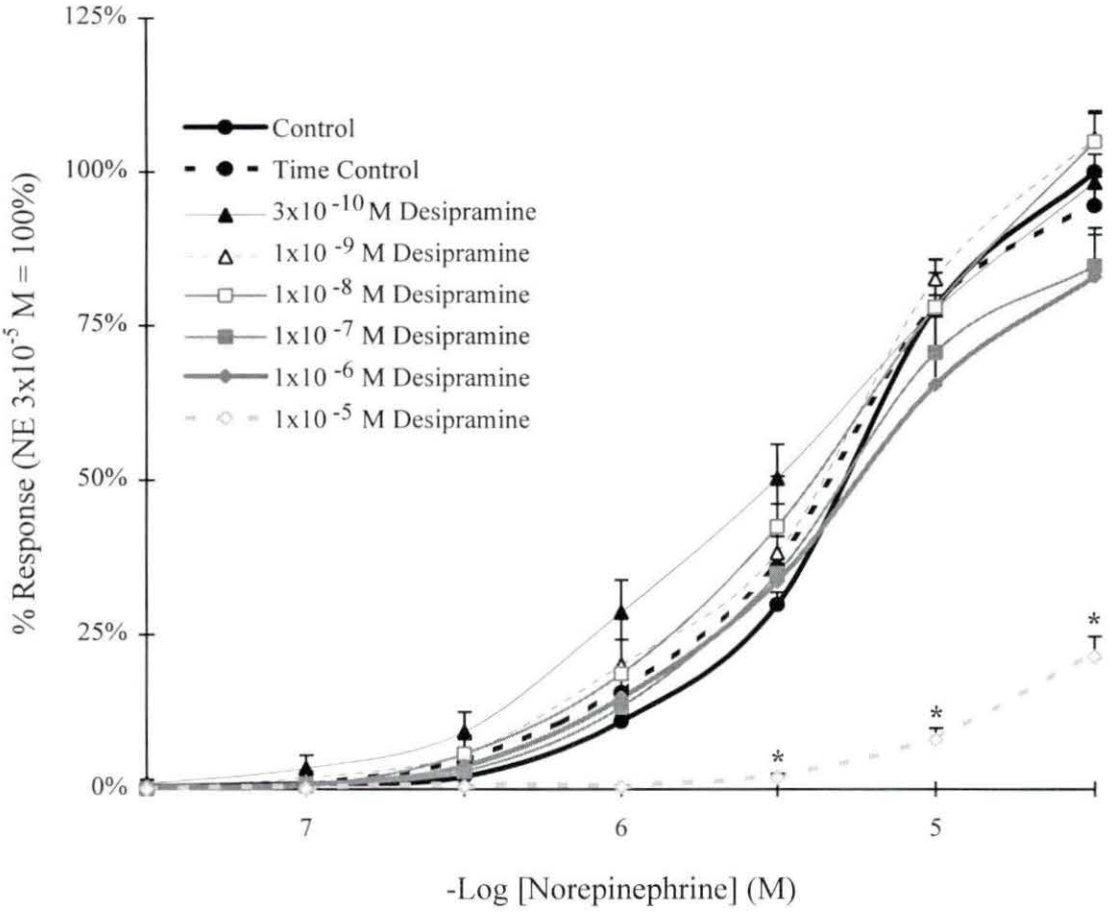

Figure 4.5. The effect of desipramine pretreatment (30 minutes) on the contractile response to norepinephrine. An asterisk (*) indicates points on the curves that are significantly different from the time control response. Each data point represents the mean ± S.E. for 5 animals.

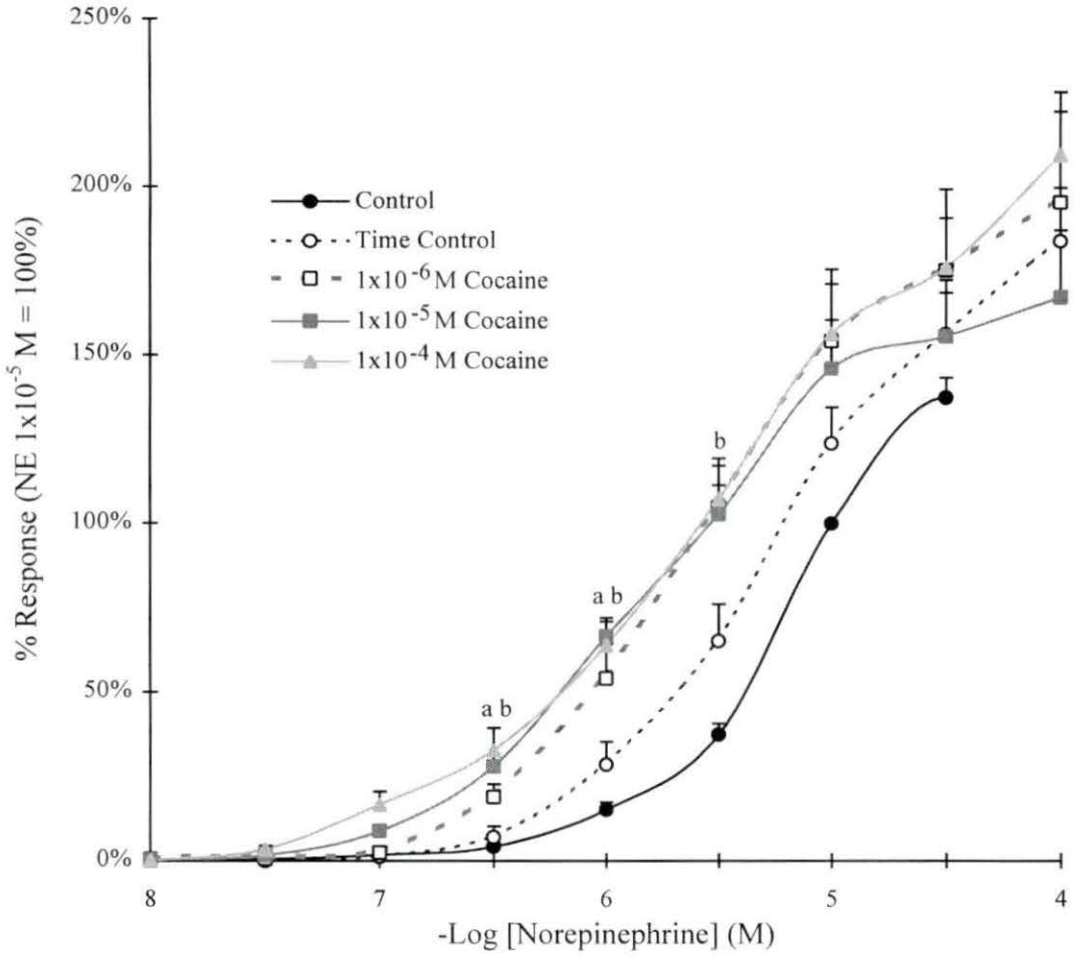

Figure 4.6. The effect of cocaine pretreatment (30 minutes) on the contractile response to norepinephrine. Letters (a = $1x10^{-5}$ M cocaine, b = $1x10^{-4}$ M cocaine) indicate points on the curves that are significantly different from the time control response. Each data point represents the mean ± S.E. for 5-6 animals.

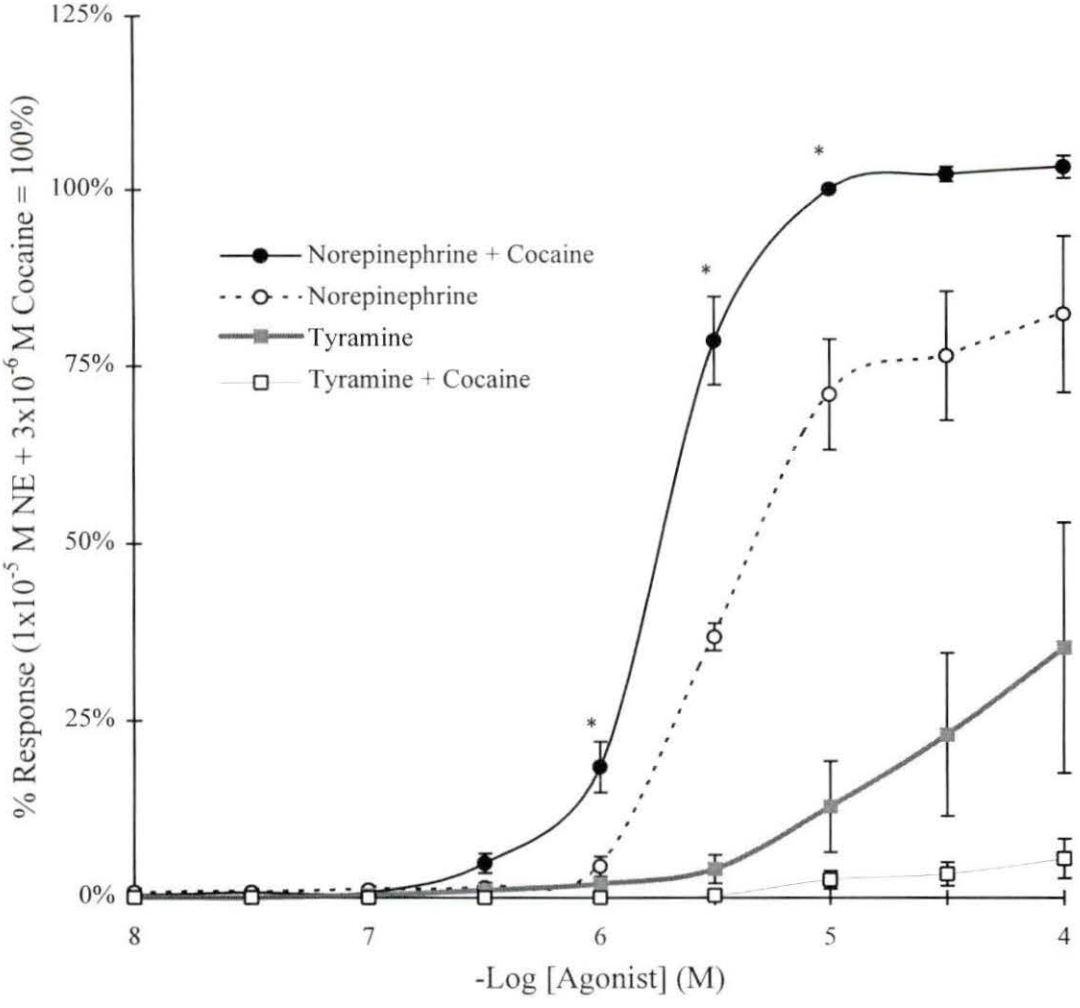

Figure 4.7. The concentration-response relationship for norepinephrine and tyramine in the presence and absence of 3μM cocaine. A control response was initially determined to 10μM NE in the presence of 3μM cocaine on all four tissues. Following washout, two of the tissues were pretreated with 3μM cocaine (20 minutes). This was followed by the generation of a concentration-response relationship for both agonists in the presence and absence of cocaine. An asterisk (*) indicates points on the curve that are significantly different from the response to norepinephrine in the absence of cocaine. Each data point represents the mean ± S.E. for 4 animals.

to significantly potentiate the response to NE (n=4). In these iproniazid treated tissues, tyramine elicited a maximum response that was 40% and 34% of the maximum response to NE in the absence and presence of cocaine, respectively. The experimental design of the present study assumed that the control response to NE, in the presence of cocaine, was completely washed out of the tissue before the subsequent procedures were carried out. There is a possibility that cocaine was not completely washed out of the tissue after the determination of the control response. However, the results do not suggest this since the control response was not significantly different from the response to NE, in the presence cocaine, when determining the tyramine and NE concentration-response relationships.

The role of the endothelium in vasoactivity

The endothelium was removed by rubbing the lumen of the tissues with a toothpick (n=4). This did not significantly increase the maximum tension developed (g) to NE or shift the EC_{50} of the NE concentration-response curve to the left (Figure 4.8). Coincidentally, inhibiting the synthesis of NO with L-NAME also did not significantly effect the maximum tension developed (g) to NE or shift the EC_{50} of the NE concentration-response curve to the left (Figure 4.9). Acetylcholine relaxed arteries contracted by KCl (45mM), addition of sodium nitroprusside (10μM) further relaxed the contraction by approximately 15%. Pretreatment with L-NAME (100μM; n=4) inhibited the acetylcholine-induced relaxation (Figure 4.10).

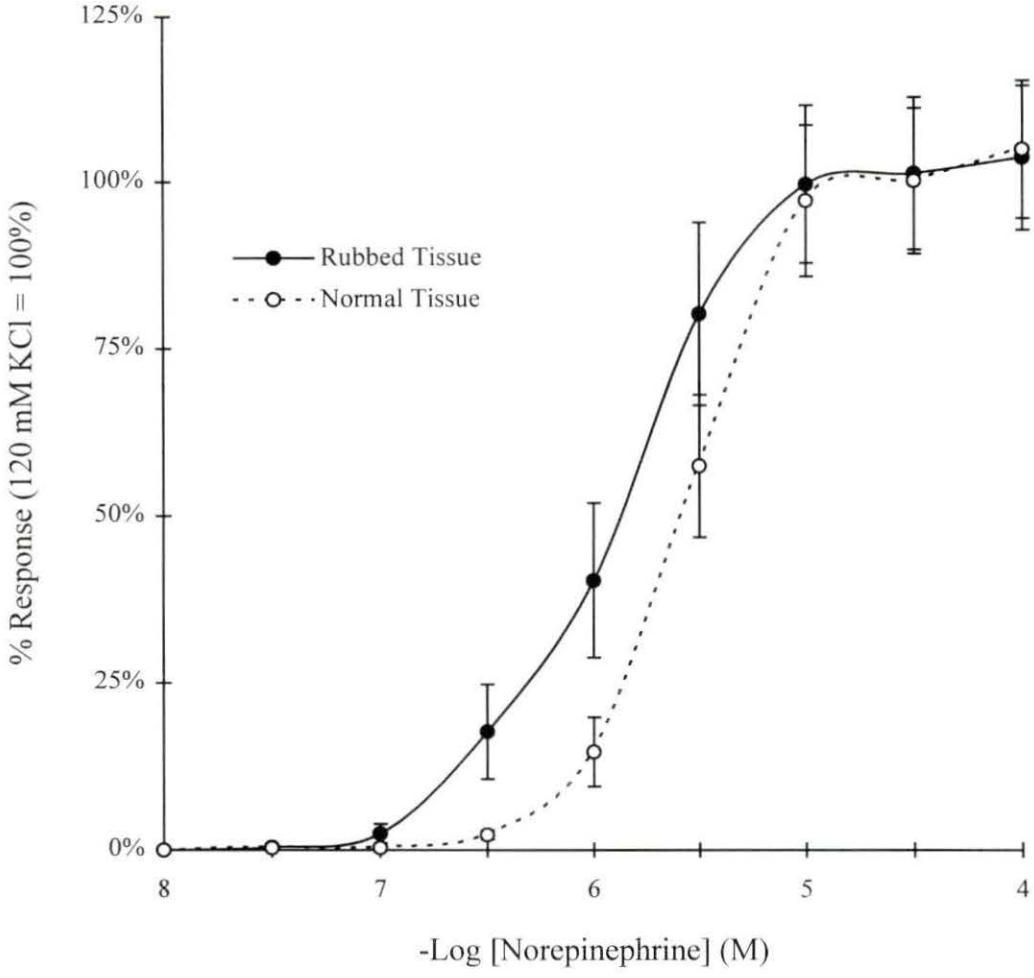

Figure 4.8. The response to norepinephrine in the presence and absence of an endothelium. To remove the endothelium, the lumen of the tissues were rubbed using a toothpick. A control response to 120 mM KCl was initially determined before generating a norepinephrine concentration-response relationship. Each data point represents the mean ± S.E. for 4 animals.

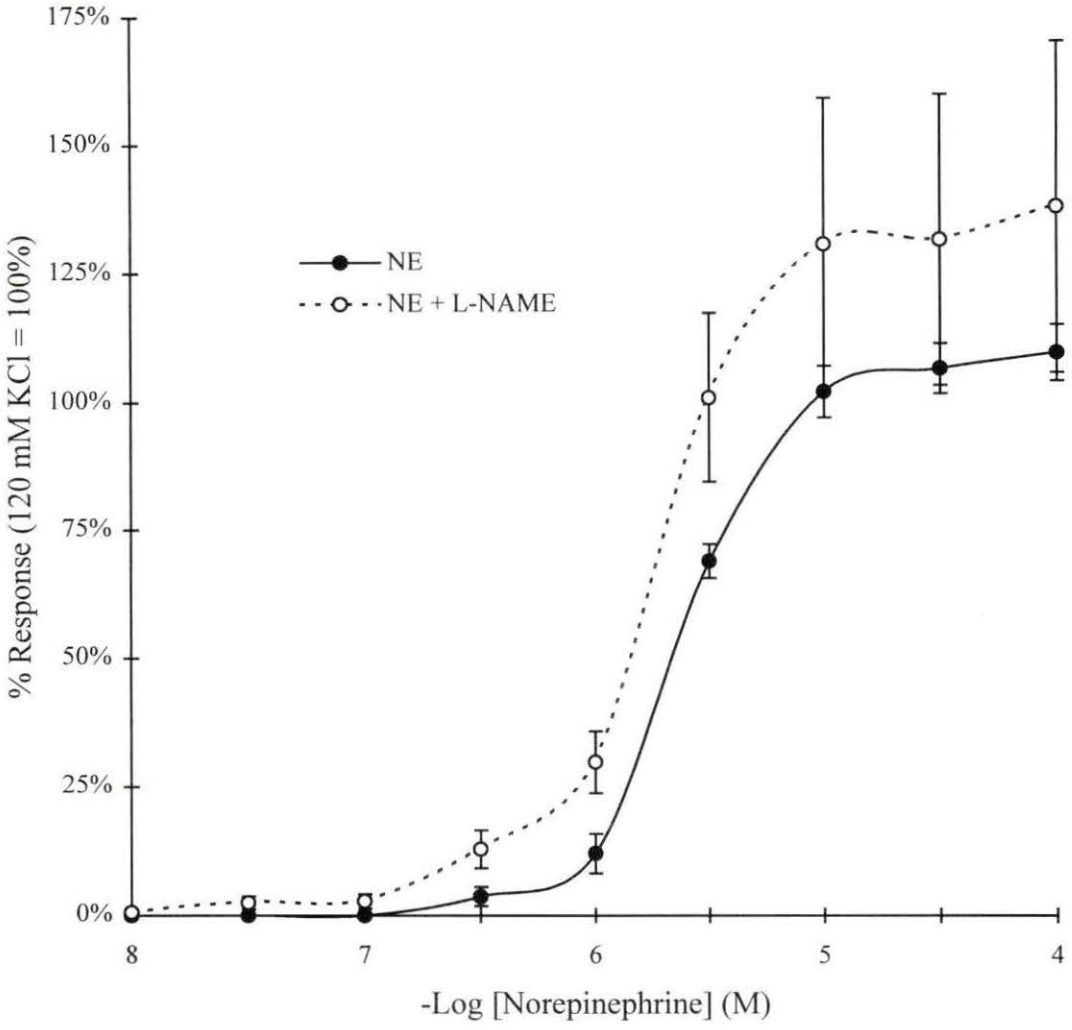

Figure 4.9. The effect of L-NAME (100μM) pretreatment (30 minutes) on the contractile response to norepinephrine. A control response to 120 mM KCl was initially determined on all tissues before generating a norepinephrine (NE) concentration-response relationship. Each data point represents the mean ± S.E. for 4 animals.

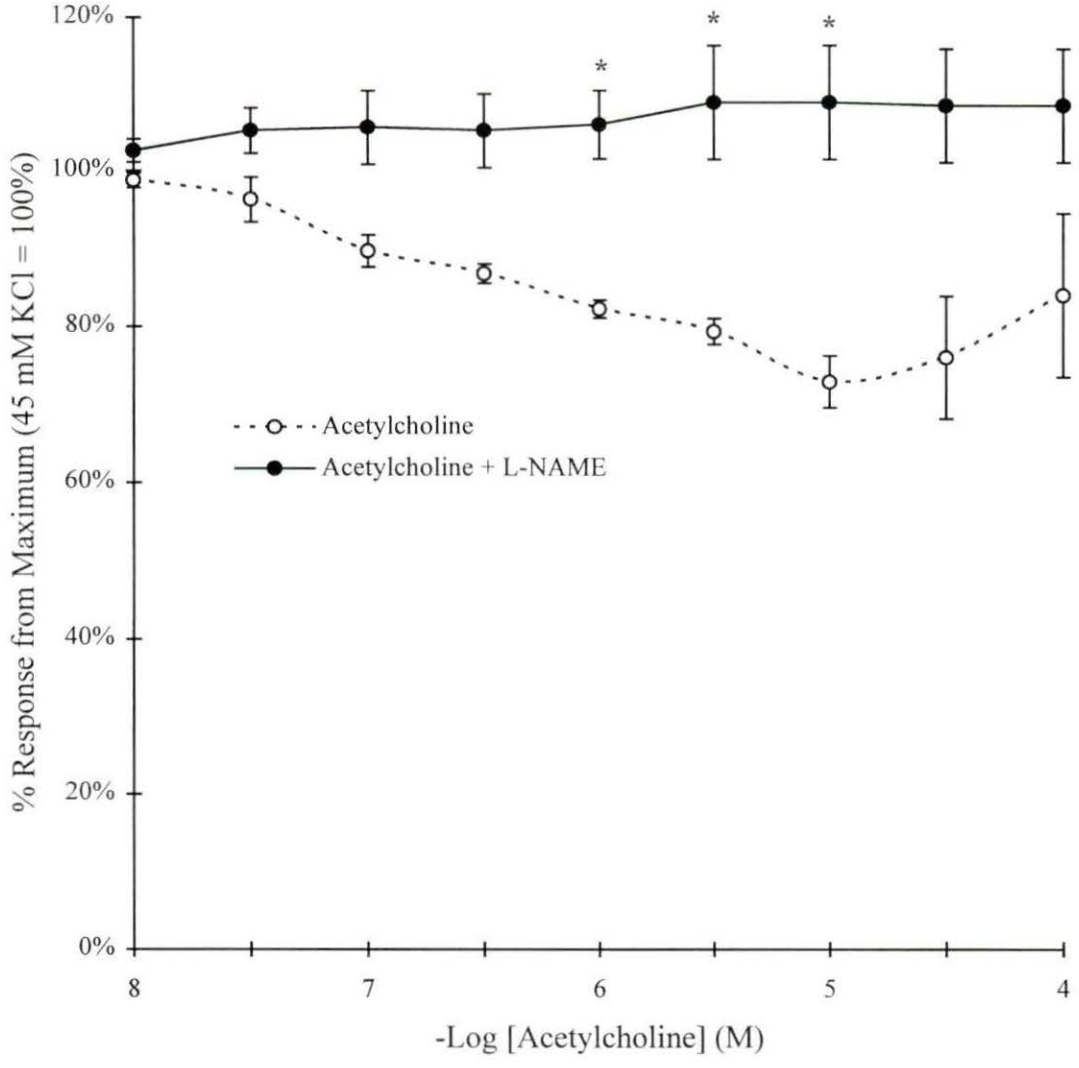

Figure 4.10. Relaxation to acetylcholine in the absence and presence of L-NAME (100μM) pretreatment (30 minutes). All tissues were initially contracted with 45mM KCl before determining the acetylcholine concentration-response relationship. An asterisk (*) indicates points on the curve that are significantly different from the response to acetylcholine in the absence of L-NAME. Each data point represents the mean ± S.E. for 4 animals.

Comparison of the contractile response to several agonists

The maximum response and potency (EC_{50}) produced by several agonists were compared. The maximum contractile response elicited by these agonists from highest to lowest was as follows: epinephrine > norepinephrine > serotonin > phenylephrine > angiotensin II > medetomidine (Table 4.1). The α_2-adrenoceptor agonist, B-HT 920, failed to produce a contraction (0.01-100μM). The potency of the various agonists was calculated at the EC_{50} of their respective concentration-response curves (Figure 4.11). From highest to lowest, the order of potency was as follows: angiotensin II > serotonin > norepinephrine > medetomidine > epinephrine > phenylephrine (Table 4.1).

Determination of the dissociation constant (K_A) for norepinephrine

The fraction of α-adrenoceptors remaining, q, after irreversible α-adrenoceptor inactivation with dibenamine (0.3μM) was calculated from the slope of the double reciprocal plot of equi-effective concentrations of NE before and after dibenamine treatment (n=5; Figure 4.12). The calculated q value was 0.26, indicating 26% of the α-adrenoceptors remained after dibenamine treatment. α-Adrenoceptor inactivation with dibenamine decreased the tissue response to NE by 64%. Several tissues contain an excess number of receptors which must be occupied in order to produce a maximum response. This excess of receptors above that needed to produce the maximum response constitute the receptor reserve (Ruffolo, 1982).

Table 4.1. Comparison of agonist-induced contractile responses.

Agonist	pD_2 (-log EC_{50})	Maximum Response (%)
Angiotensin II	7.77	64 ± 16
Serotonin	6.59	90 ± 16
Norepinephrine	6.16	117 ± 24
Medetomidine	5.61	46 ± 9
Epinephrine	5.16	139 ± 34
Phenylephrine	4.70	87 ± 15

The maximum response is expressed as a percentage of the response to 120mM KCl.
The data are expressed as the mean ± S.E. (n=5).
B-HT 920 failed to produce a response (n=3).

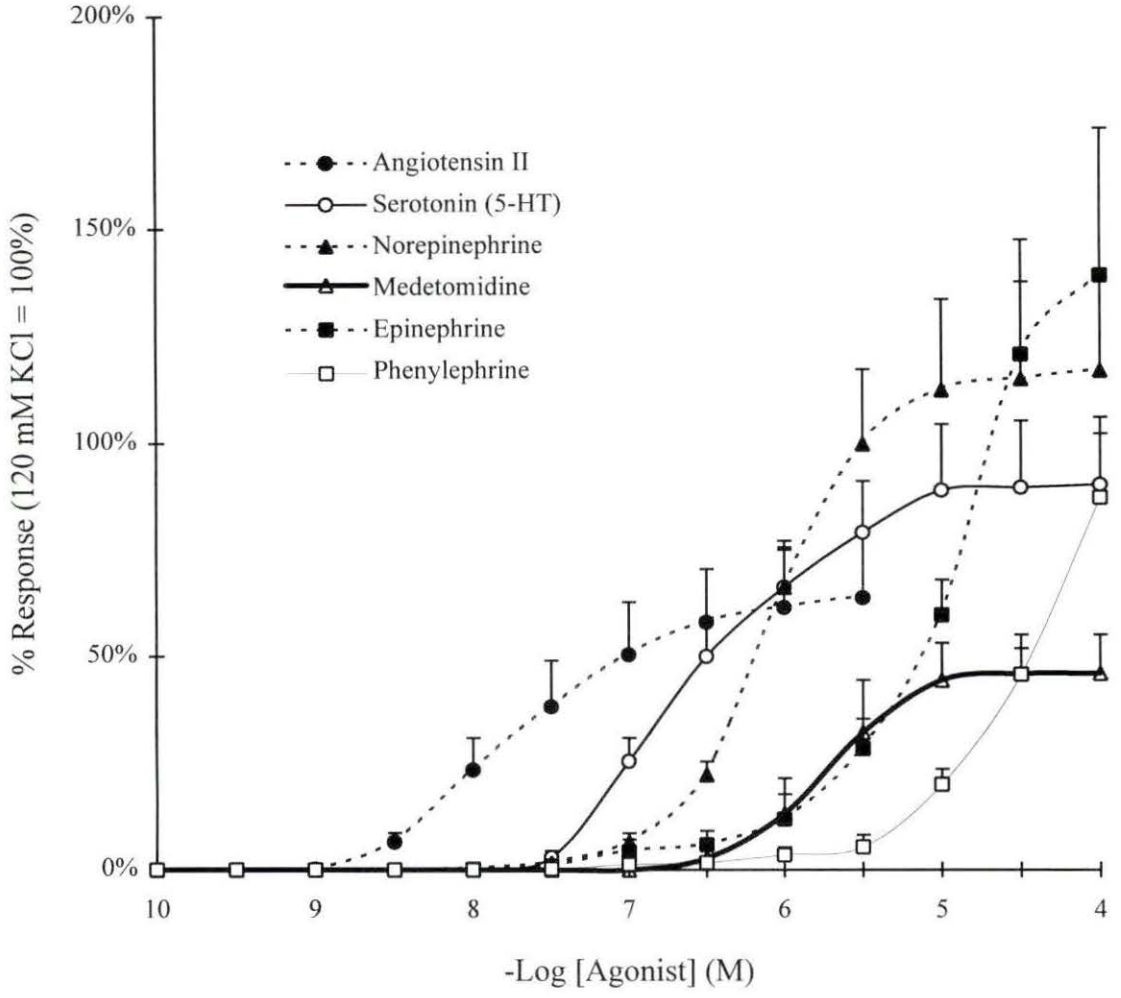

Figure 4.11. Comparison of agonists to induce a contraction. A control response to 120 mM KCl was determined on all tissues before generating the agonist concentration-response relationship. Each data point represents the mean ± S.E. for 5 animals.

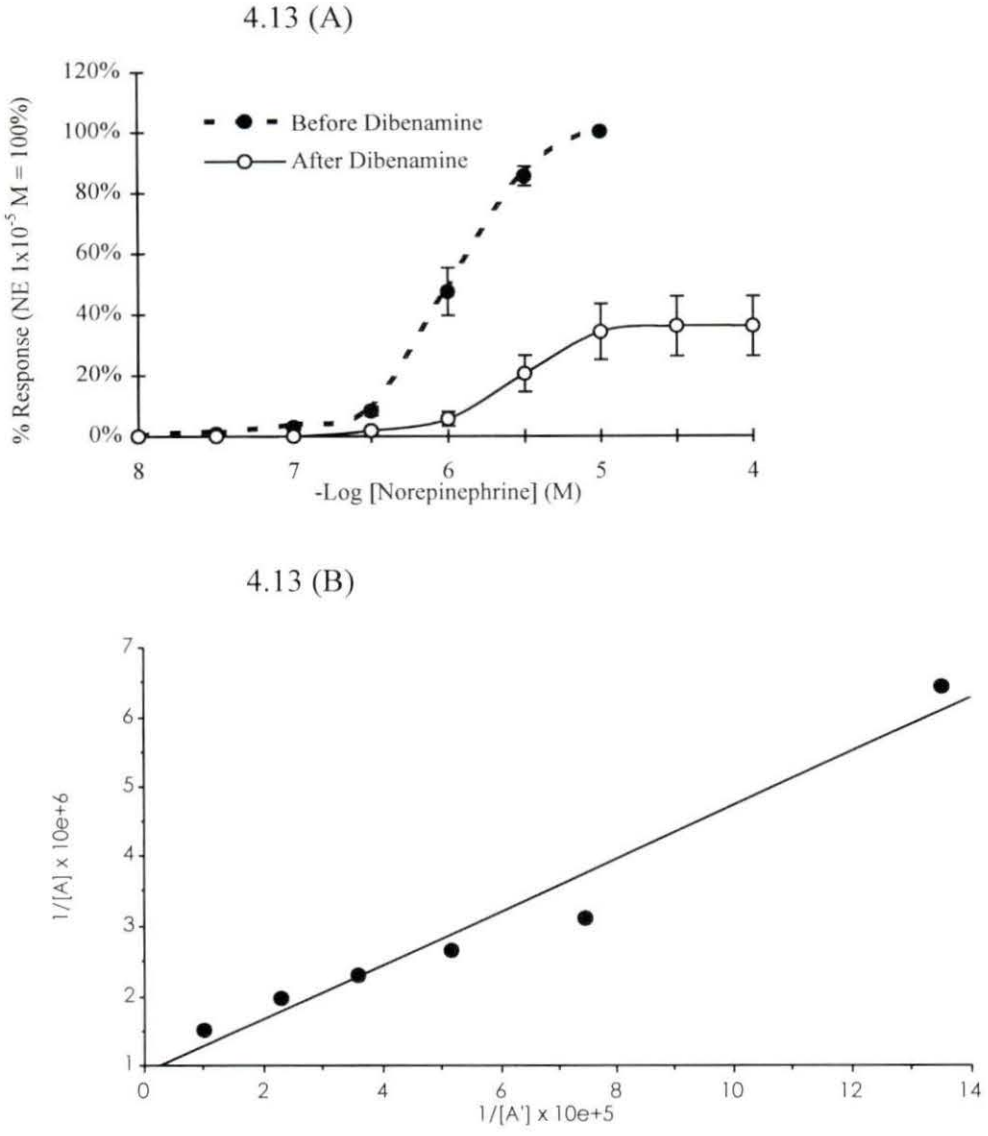

Figure 4.12. Determination of the dissociation constant for norepinephrine. (A) The contractile response to norepinephrine before and after treatment (20 minutes) with 3μM dibenamine. (B) Double reciprocal plot of equi-effective concentrations of norepinephrine before (1/[A]) and after (1/[A']) exposure to 3μM dibenamine. Each data point represents the mean ± S.E. for 5 animals.

The calculated NE dissociation constant (K_A=3.11μM) was higher than the NE EC_{50} (1.14μM). The ratio of K_A/EC_{50} (2.73) indicated that an α-adrenoceptor reserve exists in the bovine median caudal artery. According to Ruffolo (1982), the difference between the K_A value and NE EC_{50} (as the K_A/EC_{50} becomes greater than 1) reflects the existence and magnitude of the α-adrenoceptor reserve. The percentage of adrenoceptors that need to be occupied to generate a half-maximal response was calculated using the ratio EC_{50}/K_A, which is equal to 0.37 (Ruffolo, 1982). It can be concluded that 3.11μM NE bound 50% of the α-adrenoceptors, but NE only needed to occupy 37% of the α-adrenoceptors to achieve a half maximal response. This 37% receptor occupancy was achieved at 1.14μM NE.

Determination of the dissociation constants (K_B) for α-adrenoceptor antagonists

Phentolamine, the nonselective α-adrenoceptor antagonist, shifted the NE concentration-response curve to the right in a concentration-dependent fashion (n=6; Figure 4.13). The slope (-1.05) of the Schild plot (Figure 4.14) was not significantly different from unity indicating that the antagonism by phentolamine was competitive. The calculated pA_2 and K_B values were 7.36 and 43.65nM, respectively.

Prazosin, a selective α_1-adrenoceptor antagonist, shifted the NE concentration-response curve to the right at concentrations of 3, 10, 30, and 100nM (n=5; Figure 4.15). When the data were subjected to Schild plot analysis (Figure 4.16), the slope was -0.99 which

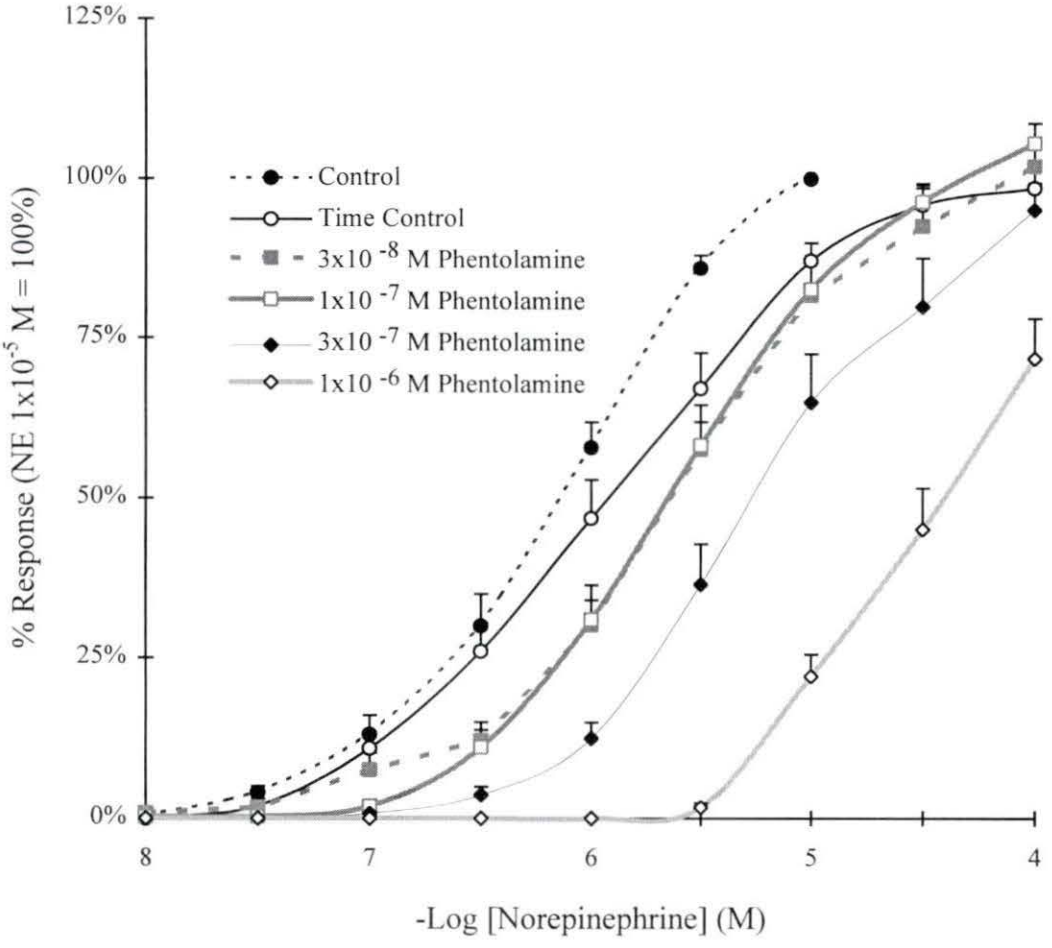

Figure 4.13. The effect of phentolamine pretreatment (60 minutes) on the contractile response to norepinephrine. Each data point represents the mean ± S.E. for 6 animals.

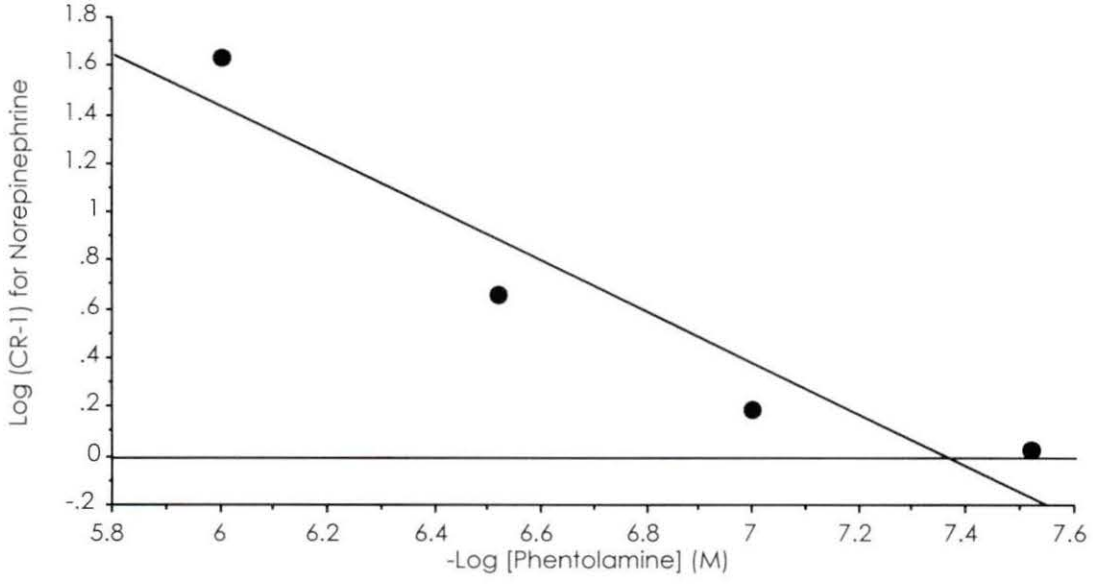

Figure 4.14. Schild plot for phentolamine against norepinephrine. CR represents a ratio at the EC_{50} of norepinephrine after and before antagonism by various phentolamine concentrations (see Figure 4.13). The EC_{50} data was corrected for time-dependent changes before being subjected to Schild plot analysis. Each data point represents the mean CR ratio from 6 animals. The pA_2 value for phentolamine is represented by the abscissa intercept ($y = -1.05x + 7.76$; $r = 0.95$; $pA_2 = 7.36$).

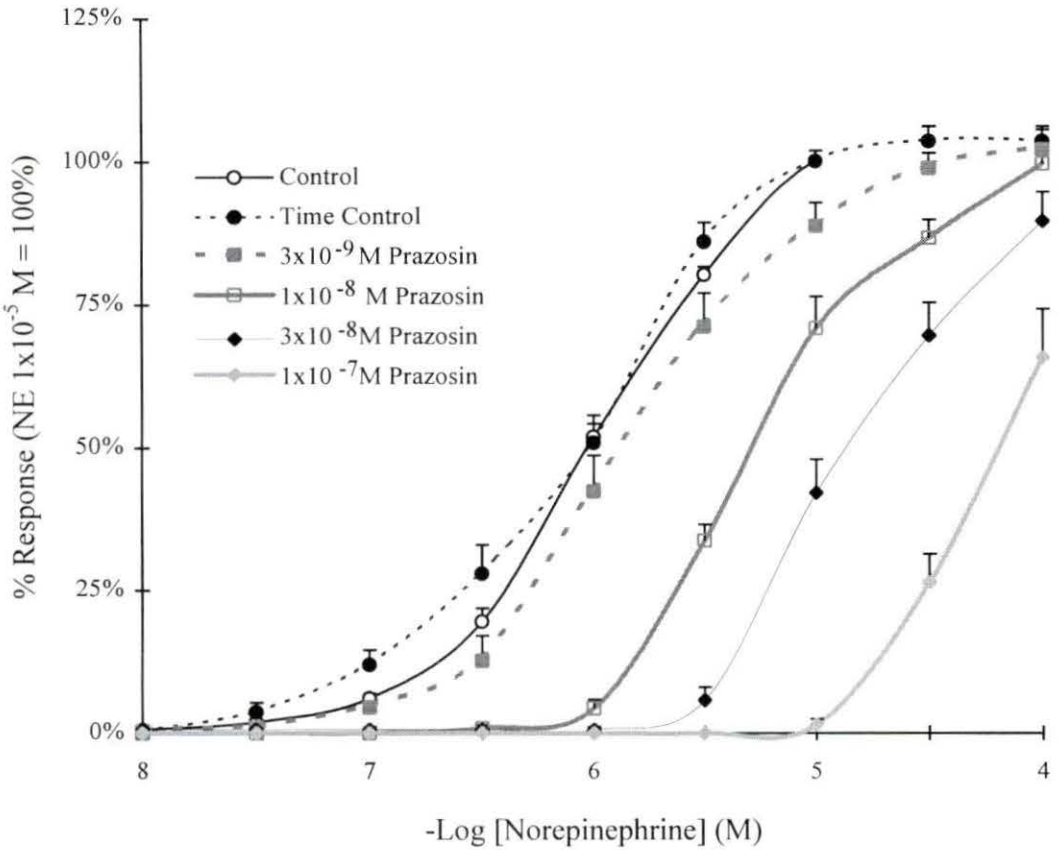

Figure 4.15. The effect of prazosin pretreatment (60 minutes) on the contractile response to norepinephrine. Each data point represents the mean ± S.E. for 5 animals.

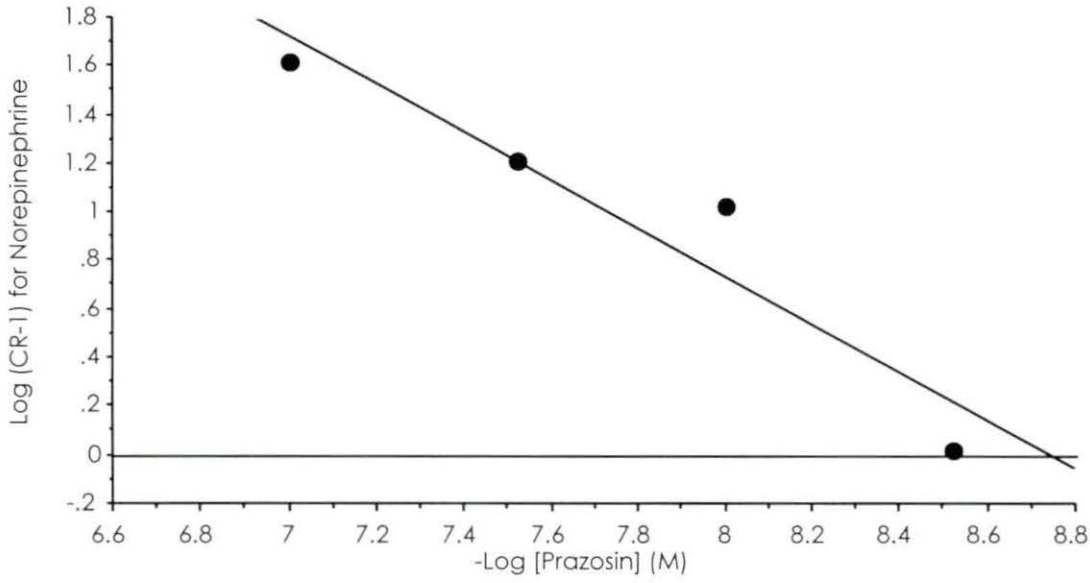

Figure 4.16. Schild plot for prazosin against norepinephrine. CR represents a ratio at the EC_{50} of norepinephrine after and before antagonism by various prazosin concentrations (see Figure 4.15). The EC_{50} data was corrected for time-dependent changes before being subjected to Schild plot analysis. Each data point represents the mean CR ratio from 5 animals. The pA_2 value for prazosin is represented by the abscissa intercept ($y = -0.99x + 8.67$; $r = 0.95$; $pA_2 = 8.74$).

was not significantly different from unity. The calculated pA_2 and K_B values for prazosin against NE were 8.74 and 1.82nM, respectively.

Prazosin was a very potent antagonist against the selective α_1-adrenoceptor agonist, phenylephrine (n=5). Incremental increases of prazosin (3-30nM) inhibited and gradually depressed the maximum response to phenylephrine (Figure 4.17). The data were analyzed at the EC_{30} instead of the EC_{50} since the response to phenylephrine in the presence of prazosin did not always reach the 50% level. The contraction was almost completely inhibited by 0.1μM prazosin (data not shown). The Schild plot (Figure 4.18) yielded pA_2 and K_B values of 9.11 and 0.78nM, respectively; the slope (-0.83) was not significantly different from unity.

Prazosin (3-30nM) depressed and shifted the concentration-response curves for the α_2-adrenoceptor agonist, medetomidine to the right. However, even though each animals' response to medetomidine was inhibited by prazosin, all the animals differed by a large magnitude in their response to medetomidine in both the presence and absence of prazosin. This large degree of variability caused the magnitudes to not be significantly different from the control response (n=6; Figure 4.19). The selective α_2-adrenoceptor antagonist, rauwolscine (3nM-100nM), was evaluated against medetomidine (n=6). No inhibition was found (data not shown). The highly selective α_2-adrenoceptor antagonist, idazoxan (RX 781094), did not alter responses to either medetomidine (Figure 4.20) or phenylephrine (Figure 4.21).

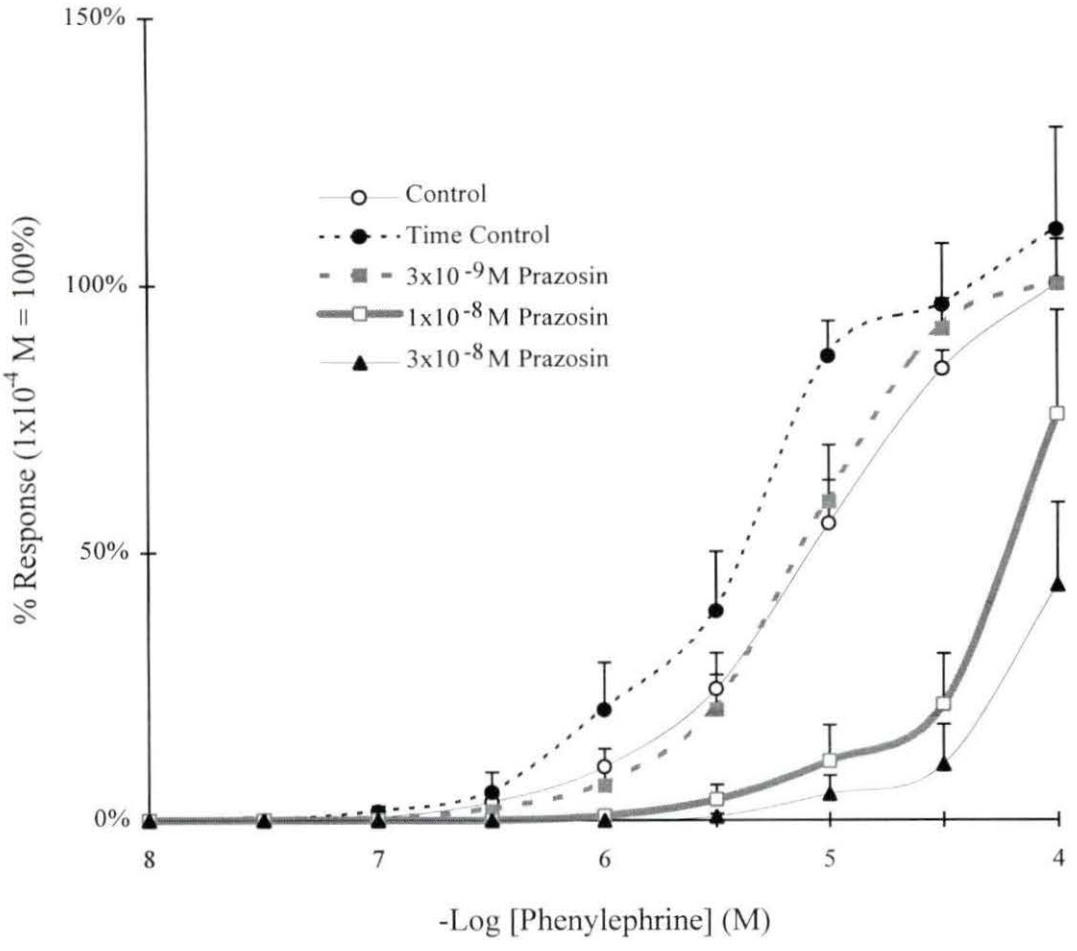

Figure 4.17. The effect of prazosin pretreatment (60 minutes) on the contractile response to phenylephrine. Each data point represents the mean ± S.E. for 5 animals.

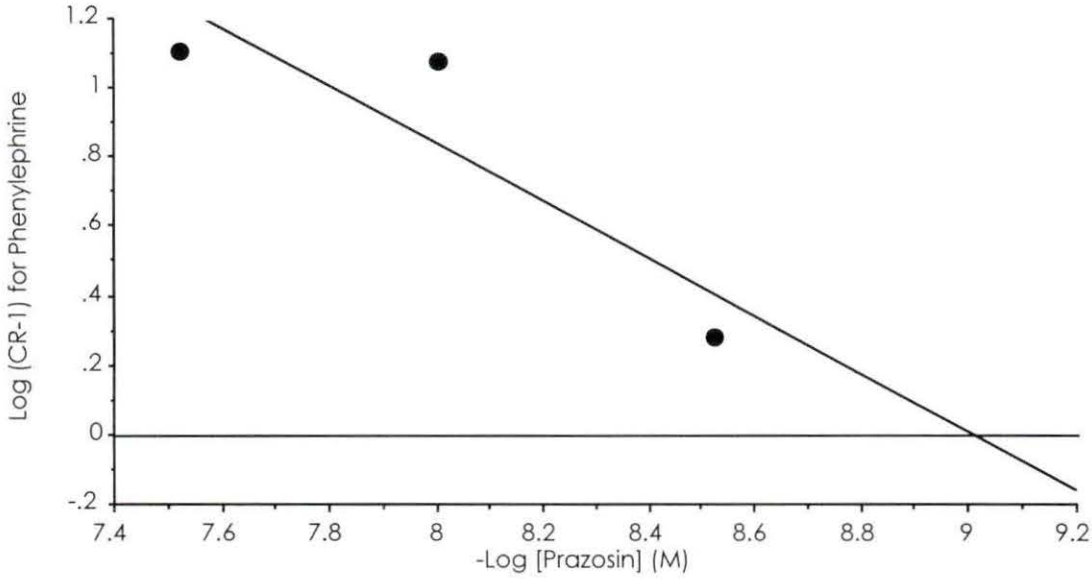

Figure 4.18. Schild plot for prazosin against phenylephrine. CR represents a ratio at the EC_{30} of phenylephrine after and before antagonism by various prazosin concentrations (see Figure 4.17). The EC_{30} data was corrected for time-dependent changes before being subjected to Schild plot analysis. Each data point represents the mean CR ratio from 5 animals. The pA_2 value for prazosin is represented by the abscissa intercept
($y = -0.83x + 7.51$; $r = 0.89$; $pA_2 = 9.04$).

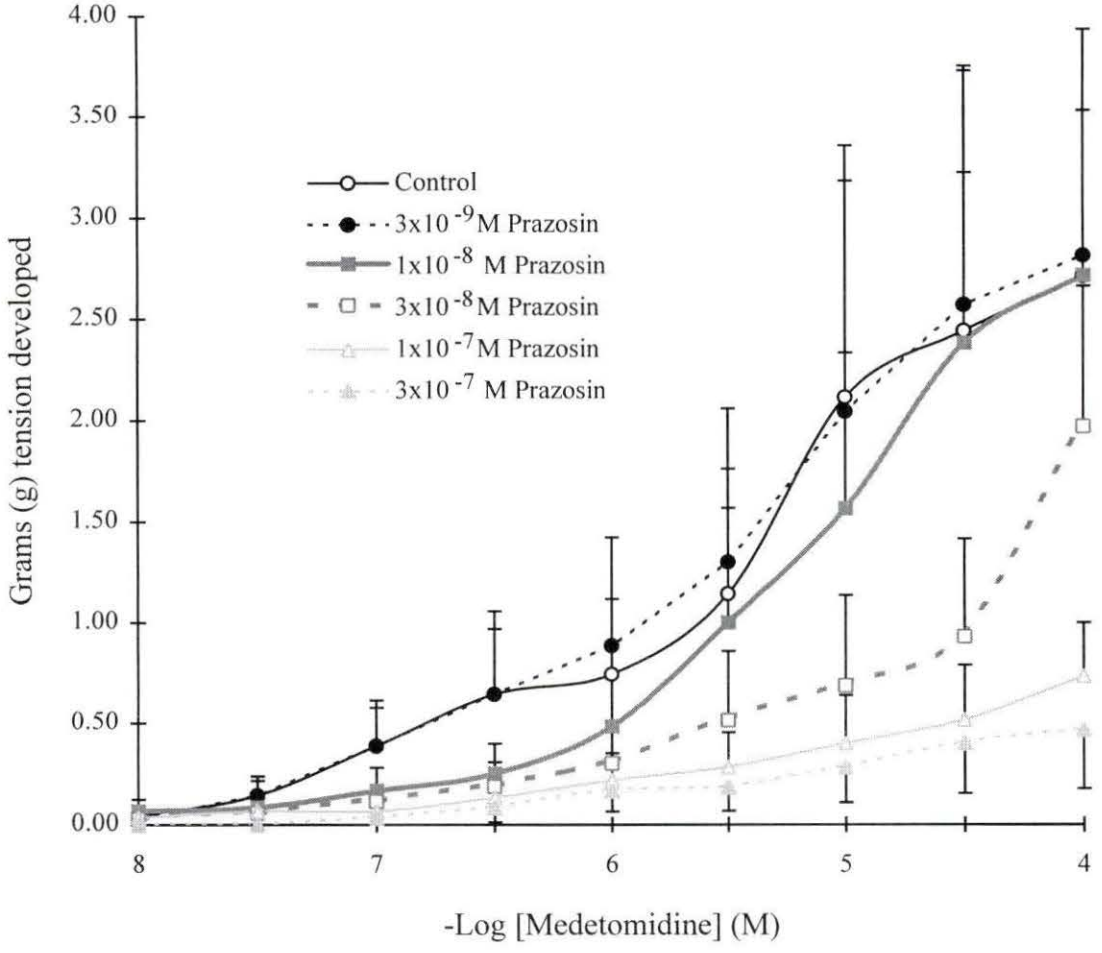

Figure 4.19. The effect of prazosin pretreatment (60 minutes) on the contractile response to medetomidine. Each data point represents the mean ± S.E. for 6-7 animals.

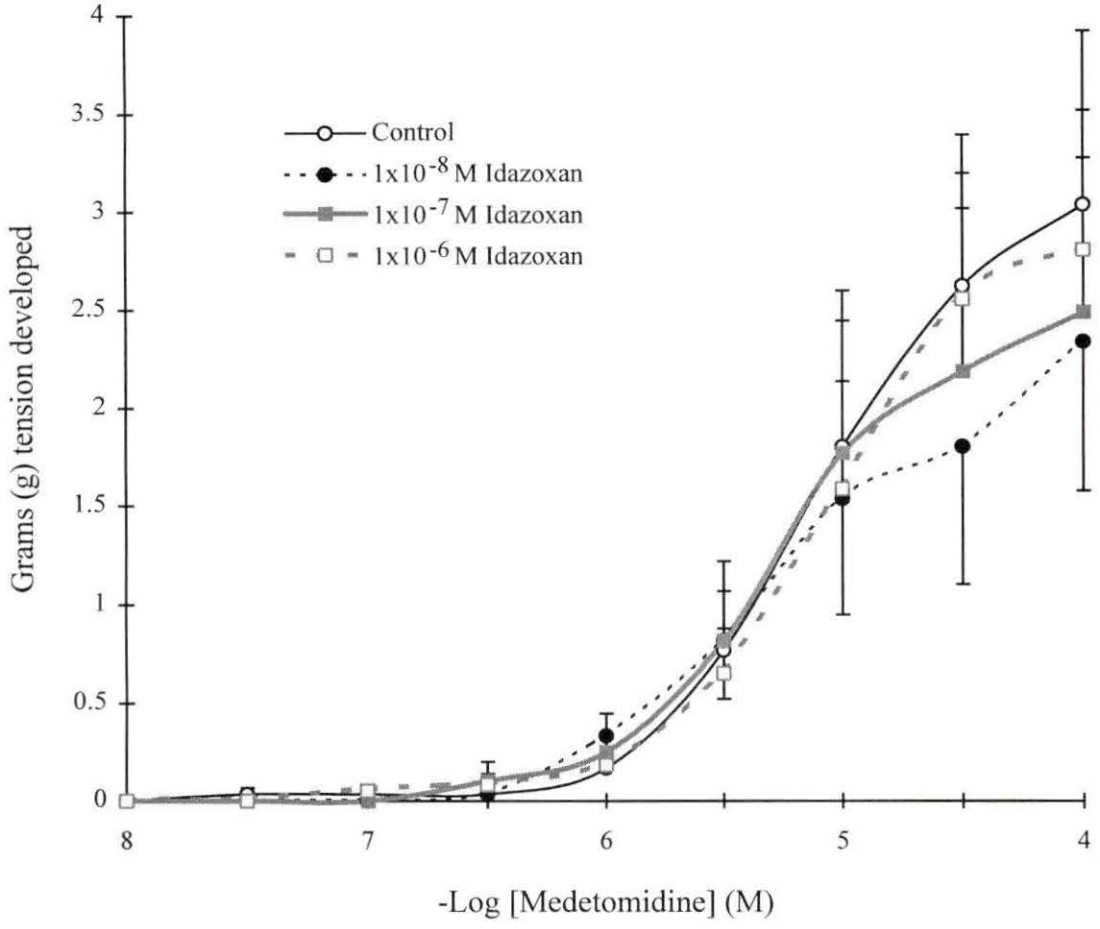

Figure 4.20. The effect of idazoxan pretreatment (60 minutes) on the contractile response to medetomidine. Each data point represents the mean ± S.E. for 6 animals.

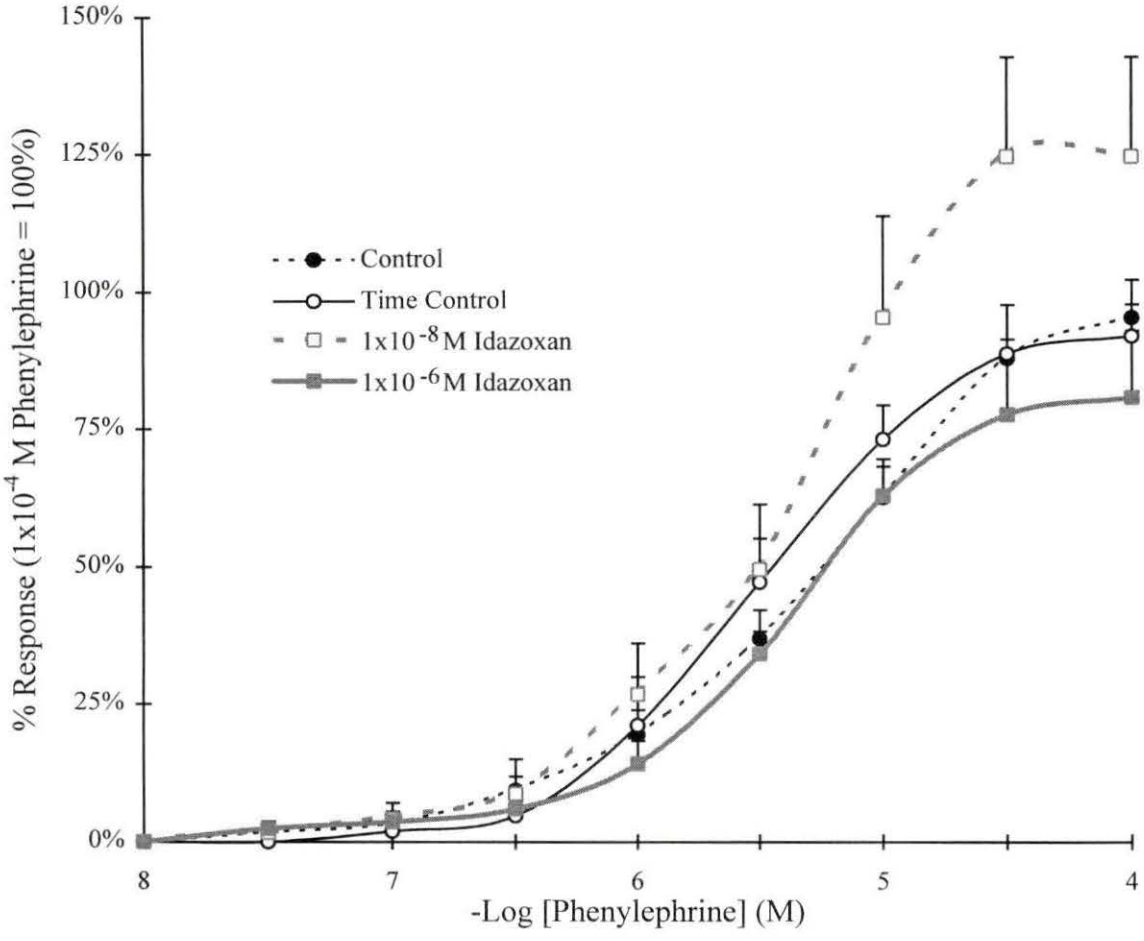

Figure 4.21. The effect of idazoxan pretreatment (60 minutes) on the contractile response to phenylephrine. Each point represents the mean ± S.E. for 5 animals.

Isoproterenol-mediated relaxation in the absence and presence of propranolol

β_2-Adrenoceptor-mediated tissue relaxation was studied using the β_1- and β_2-agonist, isoproterenol (n=5). Relaxation of the 45mM KCl-induced contraction was first observed at 0.1μM, and steadily increased with each half-log addition of isoproterenol (Figure 4.22). The maximum relaxation observed was 10.3% by 100μM isoproterenol. Isoproterenol-mediated relaxation of the KCl-induced contraction was not significantly inhibited by the β-antagonist, propranolol (n=5; Figure 4.23).

The effect of tone upon the tissue's response to medetomidine

A precontractile stimulus (approximately 4-5% of the maximum response to KCl) by either NE or phenylephrine caused a decrease in the contractile threshold for the selective α_2-adrenoceptor agonist, medetomidine, in four of the five animals studied (1 animal failed to contract to medetomidine). The sensitivity to each concentration of medetomidine with tone was not significantly different from the control response. However, at lower medetomidine concentrations the probability of response homogeneity between the control and precontractile groups decreased (Table 4.2). The medetomidine percent response values have a high degree of error since all the tissues differed from one another by more than three standard deviations. In three of the four tissues, precontraction with phenylephrine caused a greater response to low concentrations of medetomidine than precontraction with NE.

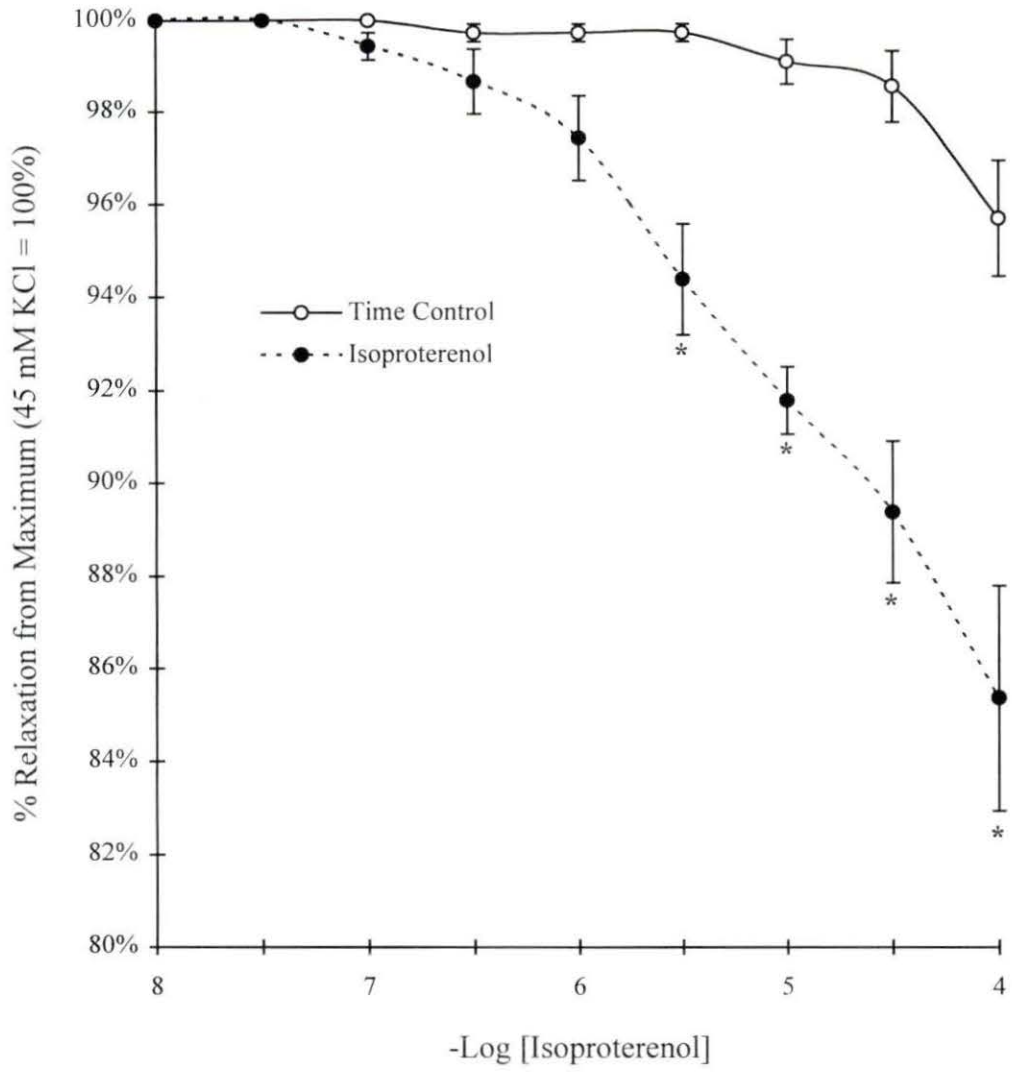

Figure 4.22. Relaxation of a KCl-induced (45mM) contraction by isoproterenol. An asterisk (*) indicates points on the curve that are significantly different from the time control response. Each data point represents the mean ± S.E. for 5 animals.

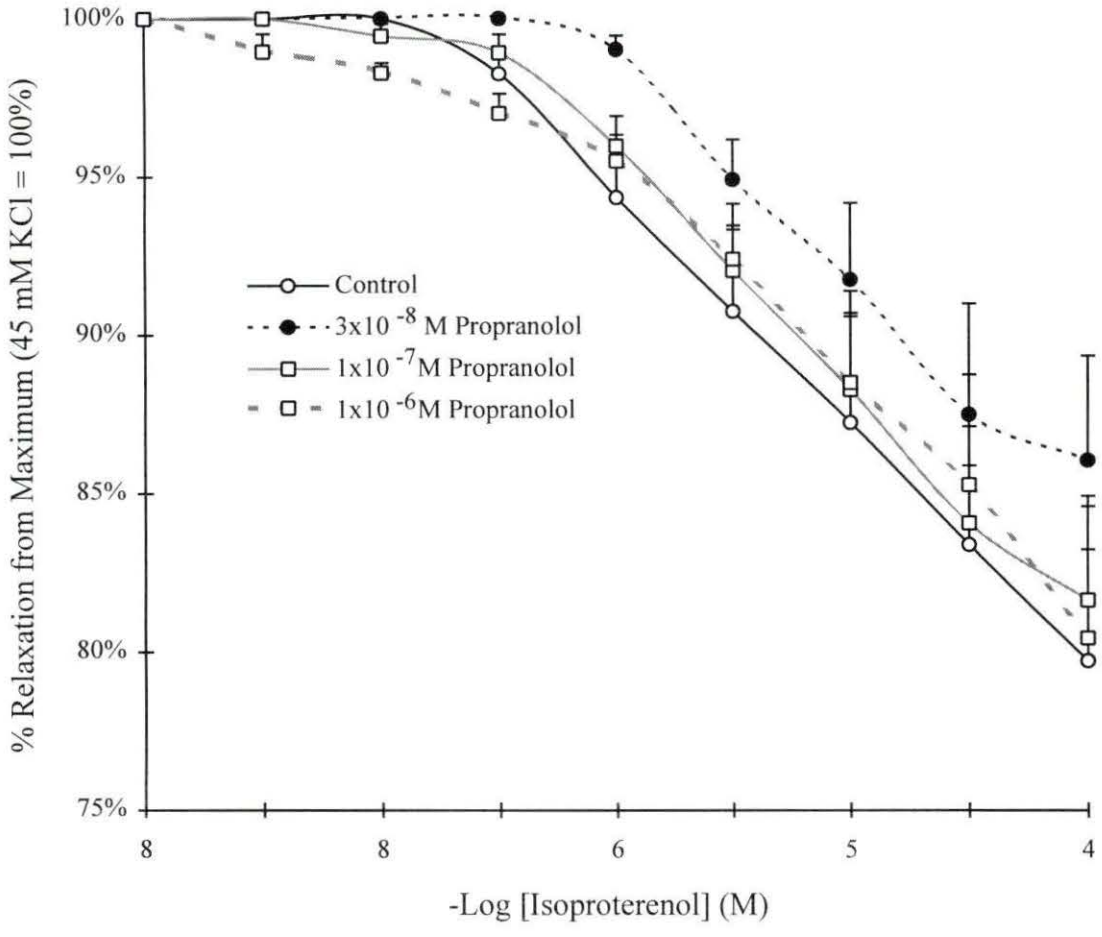

Figure 4.23. Relaxation of a KCl-induced (45mM) contraction by isoproterenol in the absence and presence of various propranolol concentrations. Each data point represents the mean ± S.E. for 5 animals.

Table 4.2. Percent response (120 mM KCl = 100%) to cumulative additions of medetomidine after an initial contraction to norepinephrine or phenylephrine.

[Medetomidine][a] (μM)	Control Mean ± S.E.M.	Norepinephrine Mean ± S.E.M.	Phenylephrine Mean ± S.E.M.	
0.01	0.25% ± 0.25	1.00% ± 1.00	1.75% ± 1.75	
0.03	0.50% ± 0.50	2.50% ± 0.02	6.25% ± 3.66	
0.1	0.75% ± 0.75	3.00% ± 3.00	10.25% ± 5.95	p = 0.08[b]
0.3	1.00% ± 1.00	5.50% ± 4.56	13.75% ± 8.25	
1	6.50% ± 5.85	14.75% ± 10.91	18.75% ± 9.86	
3	27.50% ± 22.89	19.75% ± 14.55	23.75% ± 14.48	
10	41.00% ± 20.51	25.25% ± 16.71	30.50% ± 18.14	p = 0.14[c]
30	50.75% ± 22.28	35.75% ± 19.38	34.00% ± 19.88	
100	54.50% ± 20.85	41.50% ± 19.87	35.25% ± 21.12	

The data represents 4 animals.

The mean of the response to medetomidine differed by more than 3 standard deviations.

[a] There was no significant difference in the response to individual concentrations of medetomidine between all 3 groups (ANOVA, $p > 0.05$).

[b] Probability of response homogeneity between groups for medetomidine concentrations of 0.01μM to 1μM (ANOVA).

[c] Probability of response homogeneity between groups for medetomidine concentrations of 3μM to 100μM (ANOVA).

CHAPTER 5. DISCUSSION

Catecholamine disposition mechanisms

The catecholamine concentration within the neuroeffector junction is regulated by several disposition mechanisms. Inhibition of these mechanisms should then increase the catecholamine concentration within the neuroeffector junction. Our premise stated that if the inhibited disposition mechanism plays a significant role in regulating the catecholamine concentration at the receptor site, then the sensitivity of the tissue to catecholamines would be expected to increase.

Both cocaine and desipramine were used to block the uptake$_1$ (neuronal) catecholamine disposition mechanism found within the prejuctional nerve terminal. Cocaine (10μM and 100μM) effectively potentiated the NE contractile response, while desipramine (0.3nM-10μM) failed to lower the NE EC_{50}. The three-fold shift to the left of the NE EC_{50} by cocaine (10μM and 100μM) demonstrates that the neuronal uptake mechanism is involved in catecholamine inactivation in the bovine median caudal artery. Other authors have reported similiar findings using relaxation and potentiation techniques (Trendelenburg, 1972, Guimarães and Paiva, 1977; Kalsner and Nickerson, 1969; Shibata et al., 1971). Investigators have reported that desipramine (0.1μM) potentiates the NE response to a lesser degree than cocaine (10μM) in both the rat anococcygeal muscle (Kenakin and Beek, 1981) and the isolated rabbit aorta (Auguet et al., 1982). In fact, higher concentrations of

desipramine (10μM) significantly inhibited the contractile response to NE in the present study.

Somogyi and Perel (1991) reported that when strips from the rabbit atrium where preloaded with [^{3}H]NE, high concentrations of desipramine (50-100μM) inhibited the stimulation-induced release of [^{3}H]NE, while low concentrations of desipramine (≤10μM) increased [^{3}H]NE release from the neuron. The experimental methodology used by investigators suggests that higher concentrations of cocaine (30-100μM) and desipramine (0.5-10μM) need to be used to increase the simulation-induced release of tissue-loaded [^{3}H]NE than is needed to potentiate the tissue response to NE when the amine is applied to the organ bath (Purdy et al., 1977; Hensling et al., 1983; Somogyi and Percel, 1991; Al-Damluj et al., 1993; Kenakin and Beek, 1981; Auguet et al., 1982). Therefore, 10μM desipramine may be capable of inhibiting the NE response in other tissues besides the bovine tail artery, while not inhibiting the release of [^{3}H]NE from preloaded tissues. These results suggest that despiramine's ability to potentiate the NE response, by blocking uptake$_1$, is counteracted by some inhibitory mechanism. Somogyi and Percel (1991) suggested that high concentrations of desipramine may block the adrenergic neuron similiar to the actions of guanethidine.

Cocaine caused periodic contractions in both fresh and cold stored tissues. This response has also been reported by other investigators (Egashira et al., 1991; Webb and Vanhoutte, 1982; Shibata et al., 1971; Furchgott et al., 1963). The mechanism behind cocaine-induced contractions is uncertain. Webb and Vanhoutte (1982), and Egashira et al.

(1991) suggested that cocaine-induced contractions are caused by the presynaptic release of NE. This proposal contradicts Shibata et al. (1971) who demonstrated that cold storage drastically reduces the NE content in the rabbit aorta, but does not reduce the cocaine-induced potentiation of the NE response. Studies by Wyse (1974; 1976) demonstrated that if cocaine does exhibit postjunctional effects, its contribution in potentiating the catecholamine response is very small. Bevan and Verity (1967) also indicated that cocaine does not have postjunctional effects since when the nerve terminals have degenerated, due to surgical sympathectomy, cocaine did not potentiate the NE response. The influx of extracellular calcium may be involved in the cocaine-induced potentiation of the NE response since a calcium-free media abolished the potentiation (Shibata et al., 1971). Therefore, cocaine-induced potentiation of responses to NE may involve calcium influx as well as inhibition of uptake$_1$. This idea is strengthened by the fact that in the bovine tail artery, gated calcium channels (not the sarcoplasmic reticulum) are the predominant means for increasing the intracellular calcium concentration in the contraction process (Ashida et al., 1988). Blocking calcium-dependent calcium channels decreased cocaine-induced potentiation of the response to NE (Suzuki et al., 1990). This suggests that the potentiation of the catecholamine response by cocaine is partially due to calcium influx.

Contractions to NE and tyramine in the presence and absence of cocaine confirmed the importance of uptake$_1$ in regulating the catecholamine concentration at the receptor site. Tyramine acts through uptake$_1$ to release NE from prejunctional storage vesicles. The released NE then induces a contraction by activating postjunctional α-adrenoceptors.

Cocaine interfered with tyramine's ability to release the neuronally stored NE by blocking $uptake_1$ (Furchgott et al., 1963). Though not significant, cocaine decreased the maximum response to tyramine by 29%. This lack of significance is not unusual since Furchgott et al. (1963) reported that at high tyramine concentrations (>10μM) cocaine demonstrates less ability to inhibit $uptake_1$ since they compete for the same binding site. He also indicated that to achieve an optimal inhibitory effect against tyramine, these drugs should be used in proportional concentrations. This was not done in the present study since a tyramine concentration relationship (0.01-100μM) was generated in the presence of 3μM cocaine. In the present study, cocaine also potentiated the response to NE. The maximum response elicited by tyramine, in the absence of cocaine, was approximately one-third of the maximal response to NE in the absence of cocaine. These results compare favorably with results obtained by other investigators who demonstrated that tyramine acts through $uptake_1$ to release presynaptically stored NE (Joiner et al., 1975; Auguet et al., 1982).

$Uptake_2$, found within the postjunctional cell, was studied using the $uptake_2$ inhibitor, corticosterone 21-acetate. Corticosterone 21-acetate effectively potentiated the NE response three-fold. A similar degree of potentiation, by inhibition of $uptake_2$, has been demonstrated in the rabbit aorta (Henseling et al., 1983; Martel et al., 1993; Kalsner, 1975) and dog mesenteric artery (Guimarães and Paiva, 1977). Our results indicate that $uptake_2$ is important in the catecholamine disposition process for the median caudal artery.

Catechol-O-methyltransferase (COMT), primarily found within the effector cell, was studied using the COMT inhibitor, tropolone (Verity et al., 1972). A 1.7-fold potentiation of

the NE response (EC_{50}) by tropolone has been reported by other investigators (Levin and Furchgott, 1970; Guimarães and Paiva, 1977; Wyse, 1974). It has been suggested that saturation of the COMT enzyme is possible (Kalsner and Nickerson, 1969; Graefe and Trendelenburg, 1974) since COMT has a lower K_m than $uptake_2$. (Henseling, 1980). Hence, at low concentrations of catecholamines, the rate of O-methylation serves as a good index for $uptake_2$ activity since neither disposition mechanism is saturated (Martel et al., 1993; Graefe and Trendelenburg, 1974). It appears that in the bovine tail artery, the effector cell is significantly involved in the inactivation of catecholamines since both corticosterone and tropolone significantly potentiated the NE response.

Monoamine oxidase (MAO) has little significance in the NE degradation process, as evidenced by the lack of potentiation, when inactivated with iproniazid. Electron microscopy studies indicated a lack of mitochondria in the bovine tail artery (Ashida et al. 1988). This may partially explain the negligible potentiation of responses to NE by inhibition of MAO. The failure of iproniazid to potentiate the NE response agrees with studies done on the rabbit aorta (Kalsner and Nickerson, 1969), guinea pig atria (Furchgott and Garcia, 1968), and the rat tail artery (Wyse, 1976). Neuronal uptake is not able to saturate MAO due to its high K_m (Trendelenburg et al., 1987). However, the rate of catecholamine accumulation within storage granules may prevent potentiation with iproniazid (Furchgott and Garcia, 1968). Furchgott and Garcia (1968) found that inhibition of MAO did not potentiate the NE response in the guinea-pig atria. However, inhibition of MAO did increase the uptake of NE within the nerve terminal to a greater degree than when MAO was not inhibited. This

suggests that even though inhibition of MAO did not potentiate the NE response, it may still be important in the inactivation of catecholamines. The rate of vesicular neuronal uptake of catecholamines may be extremely high in the bovine tail artery, thus preventing potentiation of the NE response due to MAO inhibition.

The concentration of catecholamines at the receptor site is predominantly determined by the density of adrenergic innervation, the thickness of the tissue, and the distance between the prejunctional neuron and the effector cell (width of the neuromuscular junction). Since $uptake_1$ is found prejunctionally on nerve terminals, the activity of $uptake_1$ may serve as an approximation of the adrenergic innervation of a tissue (Trendelenburg, 1972). Therefore, the degree of cocaine-induced potentiation of the NE response can serve as a relative index for the tissue's density of adrenergic innervation. However, if tissues differ by a large magnitude in their neuromuscular junction width or wall thickness, then inaccurate conclusions can be made about the extent of the tissue's adrenergic innervation.

The magnitude of cocaine-induced potentiation of the NE response in the bovine median caudal artery is similar to the potentiation induced by cocaine in less innervated tissues such as the rabbit aorta and dog mesenteric artery (Burnstock et al., 1972; Hirst and Edwards, 1989). Cocaine potentiated the NE response 2- to 3-fold in these tissues (Wyse, 1974; Shibata et al., 1971; Guimarães and Paiva, 1977). The degree of potentiation is low when compared to more highly innervated tissues, such as the cat nictitating membrane, guinea-pig vas deferens, and cat spleen capsule (Merrillees et al., 1963; Trendelenburg, 1972; Fillenz and Pollard, 1976; Van Orden, III et al., 1967). Cocaine potentiated the NE response

in the guinea-pig vas deferens 8- to 14-fold (Sannomiya and De Moraes, 1981; De Moraes and Capaz, 1977). In the cat spleen capsule and nictitating membrane cocaine potentiated the NE response 20-fold, and 20- to 30-fold, respectively (Trendelenburg, 1972). This is in agreement with our premise, that the magnitude of cocaine-induced potentiation serves as an index for the tissue's density of adrenergic innervation. In this instance, the cat spleen capsule is less densely innervated that the cat nictitating membrane (Trendelenburg, 1972; Van Orden, III et al., 1967). Overall, on these highly innervated tissues, cocaine potentiated the NE response approximately 3 to 7 times greater than on the bovine tail artery.

Trendelenburg (1972) hypothesized that the activity of uptake$_1$ (and maybe the density of adrenergic innervation) is inversely proportional to the neuromuscular width distance. This was demonstrated in the nictitating membrane and vas deferens. These tissues are highly innervated and have a small neuromuscular junction width distance (Trendelenburg, 1972; Nield and Zelcer, 1982; Merrillees et al., 1963; Van Orden, III et al., 1967). Our results suggest that the 3-fold potentiation of the NE response using cocaine may partially be due to a low density of adrenergic innervation for the bovine median caudal artery, and/or to a relatively wide neuromuscular junction width distance.

After cold storage, the bovine median caudal artery's maximum response to NE significantly increased. This might be partially due to nerve degeneration, as was seen by Sannomiya and De Moraes (1979) when they denervated the guinea-pig vas deferens (bilateral postganglionic denervation). However, Langer (1974) hypothesized that the

increased response to NE was due to some unknown developmental change in the postjunctional cell brought on by the cold storage.

The role of the endothelium in vasoactivity

The endothelium can influence vascular smooth muscle tone by releasing the endothelium-derived vasodilator, NO (Ignarro et al., 1988). The literature contains documention that endothelium-denuded tissues are more sensitive to catecholamines than endothelium-intact tissues (Oriowo et al., 1987; Doggrell, 1992; MacLean et al., 1993; Kaneko and Sunano, 1993). Our results tend to confirm these reports, but the increase in sensitivity to NE on rubbed arteries was not significant. Similar to the rubbed arteries, pretreatment with L-NAME (100μM) did not significantly potentiate the response to NE. However, L-NAME did inhibit the acetylcholine-induced relaxation of endothelium-intact arterial rings, indicating that the tissue is capable of synthesizing NO. The magnitude of acetylcholine-induced relaxation may be underestmiated since Ignarro et al. (1988) found that KCl inhibits acetylcholine-induced relaxation. Addition of sodium nitroprusside to a tissue exposed to acetylcholine (100mM) caused an additional but small degree of relaxation. Ignarro et al. (1987;1988) made similar observations using sodium nitroprusside on the bovine intrapulmonary artery. Our results suggest that the endothelium contributes to the vasoactivity of the bovine median caudal artery.

Comparison of the contractile responses of several agonists

A simple pharmacological way to determine the presence and to classify different receptor types is to compare the relative potencies of different agonists. However, the problem in using these comparisons is that tissues differ in their receptor density, efficacy, and affinity for each agonist (Drew, 1985). The order of potency for several agonists in the bovine median caudal artery from highest to lowest was as follows: angiotensin II > serotonin > norepinephrine > medetomidine > epinephrine > phenylephrine. B-HT 920 failed to produce a response. This demonstrates that besides α-adrenoceptors, angiotensin II and serotonin receptors (5-HT) may also contribute to the vasoactivity and tone of the artery. Serotonin receptors (5-HT_2) have been shown to be involved with vasoconstriction in the bovine uterine artery (Dyer, 1993), bovine coronary artery (Purdy and Milburn, 1991), and in the isolated perfused bovine ear artery (Eghianruwa and Eyre, 1991).

The selective α_2-adrenoceptor agonist, medetomidine, produced a contractile response at a lower concentration than that elicited by phenylephrine. However, medetomidine's potency was very low (pD_2=5.61) when compared to its potency (pD_2=9.0) on the mouse vas deferens (Virtanen et al., 1988). On the rat anococcygeus muscle, high medetomidine concentrations caused contractions that were inhibited by both prazosin and idazoxan (Scheinin et al., 1989). These results suggest that medetomidine may act as a partial α_1- and α_2- agonist at high concentrations in the bovine tail artery. Since B-HT 920 (selective α_2-agonist) failed to produce a response, this indicates that α_2-adrenoceptors have either a low affinity and/or efficacy, or that functional α_2-adrenoceptors do not exist.

The pD_2 for NE was 6.16, this is consistant with the potency for NE on the bovine dorsal pedal vein (pD_2=6.25; Solomons et al., 1989) and the rat tail artery (pD_2=6.5; Oriowo et al., 1989). The phenylephrine pD_2 for the bovine tail artery was 4.7. This is very low when compared to pD_2 values on other tissues, such as the major arteries of the rabbit (pD_2=5.5-6.7; Oriowo et al., 1987), the bovine oviductal arteries (pD_2=5.93; Costa et al., 1992), and the rat aorta (pD_2=7.27; Carrier and White, 1985). It may be that the α_1-adrenoceptors have a low affinity/efficacy for phenylephrine. There is also a possibility that a majority of the α-adrenoceptors are of the α_{1A} subtype since phenylephrine has a higher potency at the α_{1B} subtype (Bylund, 1992). This is possible since the α_{1A} subtype displays a prominant role in the peripheral circulation of animals (Piascik et al., 1994).

Epinephrine was found to be less potent than NE. However, our findings differ from studies done on other tissues. Epinephrine was found to be more potent than NE in both the rabbit aorta (Starke et al., 1975) and the rat caudal artery (Rajanayagam and Medgett, 1987; Abel and Minneman, 1986). The difference in potency between epinephrine and NE in this artery can not be explained by the presence of β_2-adrenoceptors since they were blocked by propranolol. Therefore, our data suggests that different α-adrenoceptor subtypes may exist with differences in their affinity and/or efficacy for NE and epinephrine. Since adult animals were used in this study, age might contribute to the low potency of epinephrine (McAdams and Waterfall, 1986; Jagadeesh et al., 1990) In the bovine aorta, the EC_{50} for epinephrine was 12-fold higher on aortas' from mature than young animals, even though receptor density increased with maturity. It appears that the efficacy of adrenoceptors decreases with age

(Jagadeesh et al., 1990; McAdams and Waterfall, 1986). These results suggest that the efficacy of the α-adrenoceptors may be poor for initiating the response to epinephrine in the bovine tail artery.

The adult bovine tail artery may also predominantly consist of low affinity α_1-adrenoceptor binding sites. A high and low affinity binding site for α_1-adrenoceptors has been proposed by many investigators based on the ability for competitive agonists/antagonists to displace [^{3}H]Prazosin from the α_1-adrenoceptor binding site (Morrow and Creese, 1986; Colucci et al., 1985; Jagadeesh and Deth, 1987; Minneman, 1988). These two binding sites appear to utilize different second messenger pathways for initiating the biological response (Jagadeesh and Deth, 1987; Jagadeesh et al., 1990). The low affinity site is less efficient than the high affinity site in mediating a response, since its second messenger pathway operates independent from GTP-binding to the G-protein subunit. Jagadeesh et al. (1990) reported an absence of the high affinity site in the aorta of adult bovine.

Jagadeesh and Deth (1987) found that in the aorta from young bovine, epinephrine was more potent at low affinity sites, while phenylephrine was more potent at the high affinity sites. If the bovine tail artery is primarily composed of low affinity sites, then it could be hypothesized that the concentration-response relationship would reveal a potency order of epinephrine greater than phenylephrine. This agrees with what was found for the bovine tail artery. This idea is further supported by the low pD_2 value for phenylephrine. Jagadeesh and Deth (1987) found that even though phenylephrine was more potent at the high affinity site than the low affinity site, phenylephrine had a greater affinity than both

epinephrine and NE for the low site. The difference between the potency and affinity of phenylephrine can be explained by the poor efficacy of the low site, as previously proposed by Jagadeesh and Deth (1987). Our hypothesis of a low affinity binding site does not correlate well with the method for classifying the α_1-adrenoceptor subtypes, as proposed by Morrow and Creese (1986), since phenylephrine has a low pD_2 value. Their classification method would label the high affinity site and low affinity site as being the α_{1A} and α_{1B} subtype, respectively. However, it is possible that the efficacy of the α_{1B} subtype is very low for phenylephrine. The nomenclature and method for classifying α_1-adrenoceptor subtypes has been modified since Morrow and Creese classified α_1-adrenoceptors. α_1-Adrenoceptors are now divided into three subtypes: α_{1A}, α_{1B}, and α_{1D}. It has been acknowledged that when pharmacologically classifiying α_1 subtypes, the results may display characteristics of both the α_{1A} and α_{1B} subtype. Therefore, it may not always be possible to clearly differentiate between the two subtypes (Hieble et al., 1995). Data in the literature suggests that the high affinity site can be clearly identified as the α_{1A}-subtype, but the low affinity binding site exhibits characteristics that are different from both the α_{1B} and α_{1D} subtype (Piascik et al., 1994).

α_1-Adrenoceptors mediate the release of calcium from intracellular stores in a concentration-dependent manner (Awad et al., 1983; Colucci et al., 1985). This intracellular calcium release is primarily responsible for the initial rapid phase of the contraction (Deth and Van Breemen, 1977; Ruffolo et al., 1991). Colucci et al. (1985) found that this is due to activation of the high affinity site. The bovine tail artery predominantly increases its

intracellular calcium concentration by the influx of extracellular calcium since it lacks a sarcoplasmic reticulum (Ashida et al., 1988; Goldman et al., 1989). Therefore, the contractile response of the bovine tail artery to epinephrine may not be primarily mediated by the activation of the high affinity binding site.

Our results suggest that the bovine tail artery is predominantly composed of low affinity binding sites. However, properly classifying the binding sites into subtypes can not be accurately done based on our studies. It does appear that more than one α_1-adrenoceptor binding site is present on this artery.

Evaluation of the dissociation constant (K_A) for norepinephrine

The percent of receptors occupied by an agonist is not usually proportional to the response it generates (Ruffolo, 1982). To achieve a linear relationship, 50% of the receptors would need to be occupied to obtain a half-maximal response. The double reciprocal plot of NE, before and after dibenamine treatment, indicated that 37% of the α-adrenoceptors needed to be occupied by NE to produce a half-maximal response in the bovine caudal artery. In the arteries supplying the bovine oviduct only 22% of the α-adrenoceptors needed to be occupied (Costa et al., 1992), while in the rabbit aorta only 6% of the receptors needed to be occupied to produce a half-maximal response (Besse and Furchgott, 1976). Therefore, the bovine tail artery has a limited α-adrenoceptor reserve. The K_A for NE on the bovine tail artery (3.11μM) was approximately similar to the K_A (3.95μM) for the arteries supplying the oviduct in the bovine (Costa et al., 1992). However, the NE K_A for the bovine tail artery is

somewhat higher than that reported for the rat tail artery (2.51-2.9μM) (Oriowo et al., 1989; Abel and Minneman, 1986).

The ratio of K_A/ EC_{50} is an index for the receptor coupling efficiency and receptor reserve. The greater the K_A/ EC_{50} ratio, the higher the efficacy and receptor reserve for the tissue (Ruffolo, 1982). The ratio of 2.73 on the bovine median caudal artery was similar to the 2.86 ratio on the bovine oviductal arteries (Costa et al., 1992). These ratios are low when compared to the ratio (7.9) for the rat tail artery (Oriowo et al., 1989). However, all three of these ratios are low when compared to the aorta of the cat, and the major arteries of the rabbit and rat (Oriowo et al., 1987; Oriowo et al., 1989). This suggests that the bovine median caudal artery has a small α-adrenoceptor reserve and that the receptors have a low efficacy for initiating the response to an agonist. It appears that NE has a slightly higher affinity for α-adrenoceptors in the bovine caudal artery than in the bovine oviductal artery, however the α-adrenoceptors of the bovine tail artery display a lower efficacy for initiating a response to NE. Compared with the rat tail artery, the α-adrenoceptors of the bovine tail artery have both a lower affinity and efficacy for NE. Costa et al. (1992) proposed that when activated, bovine α-adrenoceptors may be inefficiently coupled to mechanisms which trigger the biological response. An inefficient coupling mechanism was also proposed by Jagadeesh and Deth (1987) for the low affinity α_1-adrenoceptor sites.

Evaluation of the dissociation constants (K_B) for several antagonists

The use of antagonists, instead of agonists, to distinquish between receptor types is useful because it measures the affinity of the antagonist for its receptor, and is independent of the agonist used (Arunlakshana and Schild, 1959; Drew, 1985). A competitive antagonist, acting against an agonist, will yield a pA_2 value that is unique for a specific receptor type (Arunlakshana and Schild, 1959). The potent nonselective α-adrenoceptor antagonist, phentolamine, effectively shifted the NE concentration-response curve to the right. A Schild plot was used to calculate a pA_2 value for phentolamine. The pA_2 value was 7.36, which is in close agreement with the literature values (pA_2=7.48-7.8) for phentolamine acting on α-adrenoceptors (Starke et al.,1975; Ennis and Cox, 1980). The slope (-1.05) of the Schild plot did not differ from unity, indicating that the antagonism was competitive. The rat anococcygeus muscle, predominantly comprised of α_1-adrenoceptors, had a phentolamine pA_2 value of 7.70 (Drew and Whiting, 1979; Chapleo et al., 1981). These results indicate that phentolamine is acting on a population of α_1-adrenoceptors in the bovine tail artery.

Prazosin, a selective α_1-antagonist, antagonized contractions induced by NE (nonselective α-adrenoceptor agonist) and phenylephrine (selective α_1-agonist). Calculated pA_2 values for prazosin against NE and phenylephrine were 8.74 and 9.11, respectively. Prazosin's antagonism was competitive against both agonists since the slopes of the Schild plots were not significantly different from unity.

On the bovine oviductal arteries (Costa et al., 1992) and the rat caudal artery (Oriowo et al., 1989), prazosin had a pA_2 value of 9.38 and 8.8, respectively. Other investigators have

reported similiar prazosin pA_2 values (7.9-9.8) on a variety of tissue types (Dunn et al., 1991; Medgett and Langer, 1984; Isla and Dyer, 1990; Noguchi et al., 1993; Ennis and Cox, 1980; Agrawal et al., 1984). At high concentrations of prazosin the maximum response to NE was depressed and this was also observed in the human omental vein (Steen et al., 1984). The maximal contraction to phenylephrine was depressed by prazosin (10, 30, 100nM). This reduction in phenylephrine's maximum response by prazosin was similarly observed in the rat tail artery (Savino and Varela, 1991) and rat aorta (Doggrell, 1992).

Prazosin depressed and shifted the medetomidine cumulative concentration-response curves to the right. However, the data were not analyzed by Schild plot analysis since prazosin (10-300nM) prevented medetomidine from generating a sigmoidal curve with a steep linear relationship. The selective α_2-adrenoceptor antagonist, idazoxan (3nM-1000nM), did not affect the concentration-response curves for medetomidine or phenylephrine. Data in the literature suggests that idazoxan can discriminate between α_1- and α_2-adrenoceptors, with the latter predominantly antagonized at low (10nM) idazoxan concentrations (Medgett and Langer, 1984). The specificity of idazoxan for α_2-adrenoceptors was described by Chapleo et al. (1981) and Virtanen et al. (1988) in the rat vas deferens. Since prazosin, but not idazoxan, inhibited the response to medetomidine, this suggests that medetomidine is acting as a partial α_1-adrenoceptor agonist in the bovine tail artery. This is supported by the fact that rauwolscine (3nM-100nM), a selective α_2-adrenoceptor antagonist also failed to inhibit the medetomidine-induced contraction. The

studies, using idazoxan and rauwolscine, suggest that the bovine tail artery lacks functional α_2-adrenoceptors.

The effect of tone upon the tissue's response to medetomidine

The literature suggests that postjunctional α_2-adrenoceptors can be revealed and expressed by increasing vessel tone (Dunn et al., 1991; Templeton et al., 1989; Furuta et al., 1988). However, partially contracting the tissue with a low concentration of NE or phenylephrine did not significantly increase the response of the artery to medetomidine. There did appear to be a greater increase in sensitivity, though not significant, between the control and precontratile groups at low medetomidine concentrations. By placing the vessel under tone we were not able to demonstrate that α_2-adrenoceptors exist in the bovine tail artery. Tissue responsiveness to concentration-dependent increases of medetomidine varied considerably among all the arteries studied. Since Medgett and Langer (1986) hypothesized and demonstrated that the density of α_2-adrenoceptors differs among different strains of rats, it may be that individual animals possess their own unique density of α_2-adrenoceptors which maybe influenced by the animal's breed, sex, or sexual maturity.

The evaluation of the response to isoproterenol in the absence and presence of propranolol

A population of β_2-adrenoceptors appears to exist on the bovine tail artery since isoproterenol, in the presence of phentolamine, significantly relaxed arteries contracted to

KCl (45mM). However, isoproterenol only reduced the contraction by 10%. There is either a low density of β_2-adrenoceptors, or the β_2-adrenoceptors have a low affinity and/or efficacy for isoproterenol. Adenylyl cyclase can be inhibited or stimulated by α_2- and β_2-adrenoceptors, respectively. Therfore, the ability for the bovine tail artery to activate adenylyl cyclase may be poor, or adenylyl cyclase may have an ineffiecent coupling mechanism for initiating it's course of action since our results also support the absence of functional α_2-adrenoceptors (Rajanayagam and Medgett, 1987; McGrath, 1983).

Propranolol (0.03-1μM) did not inhibit isoproterenol-mediated relaxation of the artery contracted to KCl. It may be that propranolol was not allowed to equilibrate long enough in the bathing solution. However, this is unlikely since in the bovine coronary artery propranolol antagonized the isoproterenol-induced relaxation after 30 minutes of equilibration time, and the Schild plot yielded a slope of unity (Purdy and Stupecky, 1986). A KCl-induced contraction may also not be the most appropriate agonist to use to study the effect of isoproterenol. However, in the bovine intrapulmonary artery, there was no significant difference in the isoproterenol-induced relaxation between KCl- and phenylephrine-precontracted rings (Ignarro et al., 1988). Coincidentially, Purdy and Stupecky (1986) also used a KCl-induced contraction to successfully calculate a pA_2 (8.43) value for propranolol against isoproterenol in the bovine coronary artery. These results indicate that a small but inefficient population of β_2-adrenoceptors exists on the bovine median caudal artery. There may also be a small population of β_3-adrenoceptors on the artery, as was demonstrated in the canine cutaneous vasculature (Berlan et al., 1994).

CHAPTER 6. SUMMARY

Inhibitors of both the uptake$_1$ and uptake$_2$ catecholamine disposition mechanisms, and COMT, were effective in enhancing responses to NE. This suggests that uptake$_1$, uptake$_2$ and COMT are important in regulating the concentration of NE in the neuroeffector junction of the bovine median caudal artery. Monoamine oxidase appears to have little importance in the regulation of catecholamines at the α-adrenoceptor site. However, some uncertainties exist about the significance of the uptake$_1$ mechanism since cocaine, but not desipramine, potentiated the response to NE. Coicidentially, while cocaine potentiated the response to NE, cocaine also inhibited the response to tyramine. This suggests that the uptake$_1$ mechanism is intact and functional in the artery. High concentrations of desipramine (10μM) actually inhibited the response to NE. This suggests that desipramine's ability to potentiate responses to NE may be counteracted by its inhibitory action.

The importance of the endothelium in regulating vasoactivity is unclear. While inhibition of nitric oxide synthesis by L-NAME inhibited acetylcholine-induced relaxation, L-NAME did not significantly affect responses to NE, nor did removal of the endothelium.

The artery may have a population of β_2-adrenoceptors that are capable of subserving a small degree of arterial relaxation. However, since the relaxation response to isoproterenol was not inhibited by propranolol, proof of the presence and importance of β_2-adrenoceptors was not attained.

Agonist potency comparisons and the dissociation constants for phentolamine and prazosin suggest that a large functional population of α_1-adrenoceptors exists on the artery. However, the α-adrenoceptor reserve is limited. Thirty-seven percent of the receptors need to be occupied to produce a half-maximal response. As similarly concluded by Costa et al. (1992) on the bovine oviductal arteries, the α_1-adrenoceptors appear to have an inefficient coupling mechanism for contracting the bovine tail artery as revealed by the low K_A/EC_{50} ratio. Medetomidine generated a significant contraction that was inhibited by prazosin, but not by idazoxan or rauwolscine. However, the α_2-adrenoceptor population may not be functional since B-HT 920 did not generate a contraction. Medetomidine also had a low pD_2 value which was not significantly increased when the artery was put under tone. Therefore, medetomidine appears to act as a partial α_1-adrenoceptor agonist. This suggests that the bovine median caudal artery lacks functional α_2-adrenoceptors.

Some additional studies need to be done in the future. We feel that our agonist comparison contractile results are valid. However, since the response of the tissue to epinephrine contrasts a majority of the literature data, this experimental study needs to be repeated. There is still some uncertainty concerning the existance of the α_2-adrenoceptor population. Therefore, further experiments in attempting to "uncover" their presence are needed. Perhaps placing vessels under tone with other agonists, such as angiotensin II, vasopressin, serotonin, or endothelin may prove fruitful. The functional significance of the β_2-adrenoceptor population needs to be ascertained using some additional agonists to induce tone. Since only a KCl-induced contraction was used to study isoproterenol-induced

relaxation, perhaps vasopression, angiotensin II, prostaglandin $F_{2\alpha}$, or phenylephrine should by used to induce tone. Of course, blockade of the isoproterenol response by a β_2-antagonist, such as propranolol, will be needed to convice the scientific community of the presence and activity of β_2-adrenoceptors in the median caudal artery. Further experiments using radioligand binding techniques will greatly assist the pharmacological classification of adrenoceptors in this artery.

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