EFFECTS OF OVOKININ ON ISOLATED AORTAS OF GUINEA PIGS, NORMOTENSIVE AND SPONTANEOUSLY HYPERTENSIVE RATS

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(ABSTRACT)

Ovokinin, a peptide recently isolated from an enzymatic digest of ovalbumin, has been shown to mediate vasorelaxation of the canine mesenteric artery through bradykinin B1 receptors. Bradykinin can mediate both vasorelaxation and vasocontraction depending upon the tissue or species investigated. The aim of this study was to characterize ovokinin further by determining whether the effects of this peptide, like bradykinin, vary when using different species and tissue preparations as well as different contracting agents. Isolated aortic rings from guinea pigs, normotensive rats, and spontaneously hypertensive rats were exposed to phenylephrine, prostaglandin $F_{2\alpha}$, potassium chloride, or bradykinin. Bradykinin contracted guinea pig and spontaneously hypertensive rat aortas, however, it had no effect on normotensive rat aortas. In this study, ovokinin did not exhibit activity in any of the preparations except in guinea pigs, where it potentiated the contraction elicited by bradykinin only. This potentiation was blocked when rings were pretreated with captopril, a kininase II inhibitor. Ovokinin may also exhibit slight vasorelaxing activity in spontaneously hypertensive rat aortas precontracted with prostaglandin $F_{2\alpha}$. These findings suggest that, like bradykinin, the effects of ovokinin are species- and tissue-dependent. The action of ovokinin on the guinea pig aorta may involve kininase II, which is partly responsible for the degradation of bradykinin and other kinins.

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CHAPTER 1

INTRODUCTION

Ovokinin is an example of a vasoactive peptide derived from food protein (Fujita et al., 1995[a]). This octapeptide was recently isolated from an enzymatic digest of ovalbumin. It has been shown to significantly lower systolic blood pressure after administration in normotensive and spontaneously hypertensive rats. In addition, ovokinin relaxes the isolated canine mesenteric artery which has been precontracted with prostaglandin $F_{2\alpha}$. In this preparation, ovokinin appears to interact with bradykinin B1 receptors and stimulates prostacyclin release resulting in vasorelaxation. It has also been suggested that ovokinin is a weak antagonist for the angiotensin AT_1 receptor and an agonist for the AT_2 receptor. Both bradykinin and angiotensin are important contributors to the regulation of blood pressure (Unger and Gohlke, 1993 for review). This has easily been demonstrated with the success resulting from the therapeutic use of angiotensin-converting enzyme (ACE) inhibitors. ACE is responsible for the production of angiotensin II, a potent vasoconstrictor, and the degradation of bradykinin, a potent vasodilator in vivo. Thus, ovokinin, a peptide which has been suggested to interact with both bradykinin and angiotensin II receptors, may serve as an important tool in further hypertension research.

A. PRINCIPAL AIMS

Much remains to be discovered about ovokinin, and a further characterization is necessary. Bradykinin has been shown to produce different effects depending upon the species and tissue being studied. This is due to the binding of bradykinin to at least two receptor subtypes which can be coupled to several different second messenger pathways (Regoli et al., 1996). The aim of this investigation is to utilize isolated tissue preparations to determine the effects of ovokinin on a different tissue, namely aortas, from guinea pigs, normotensive and spontaneously hypertensive rats. In addition, the aortas were contracted with four different agents to see if each had an effect on the action of ovokinin.

B. LITERATURE REVIEW

1. Ovokinin

a. Isolation and Sequence Analysis

The discovery and isolation of ovokinin resulted from an attempt to find new antihypertensive proteins from enzymatic digests of food proteins (Fujita et al., 1995[a]). Isolated canine mesenteric arteries precontracted with prostaglandin $F_{2\alpha}$ were utilized to screen for such proteins. A peptic digest of ovalbumin was shown to cause vasorelaxation in the canine mesenteric artery previously contracted by prostaglandin $F_{2\alpha}$. The active peptide was separated and purified following three steps of reverse phase HPLC. The sequence was analyzed and found to share 3 residues with bradykinin and 4 with angiotensin II (Figure 1). The purified peptide was named ovokinin. Fujita et al. (1995[a]) tested the activity of a synthesized ovokinin and found it to be identical to the isolated peptide.

BRADYKININ:			R	P	P	G	F	S	P	F	R
OVOKININ:	F	R	A	¾	D	¾	H	P	F	L	
ANGIOTENSIN II:	D	R	V	Y	¾	I	H	P	F		

FIGURE 1: Amino acid sequences of bradykinin, ovokinin, and angiotensin II. A = Ala; D = Asp; F = Phe; G = Gly; H = His; I = Ile; L = Leu; P = Pro; R = Arg; S = Ser; V = Val; Y = Tyr.

b. Mechanism of Action on the Canine Mesenteric Artery

1) Receptor Determination

Because ovokinin shares 3 common residues with bradykinin, it was hypothesized that its mode of action might be mediated by a bradykinin receptor. Currently, there are

believed to be at least two subtypes of these receptors, B1 and B2. The B2 receptor appears to be responsible for the majority of the in vivo cardiovascular effects of kinins, including endothelium-dependent vasodilatation, vascular and bronchial smooth muscle contraction, and increased vascular permeability (Regoli and Barabe, 1980). Bradykinin and kallidin ([Lys^0]-bradykinin) are endogenous ligands and exhibit high affinity at these sites. A fragment of bradykinin, des-Arg^9-bradykinin, and [des-Arg^{10}]-kallidin are the ligands which bind with higher affinity at the B1 receptor. It appears that the removal of arginine at the C-terminal is necessary for good affinity at the B1 site (Marceau et al., 1997).

The effects of B1 and B2 antagonists on the activity of ovokinin were determined. (Fujita et al., 1995[a]) It was found that des-Arg^9-[Leu^8]bradykinin, a B1 antagonist, completely blocked the vasorelaxing effect of ovokinin while the B2 antagonist, Hoe 140, had no effect. In addition, binding assays were conducted using RAW264.7 cells (macrophage-like cells) for B1 receptor binding and guinea pig ileum membranes for B2 binding. The latter is known to be a sensitive B2 preparation. Ovokinin inhibited the binding of a B1 selective ligand, [^{3}H][des-Arg^{10}]kallidin (IC_{50} = 64 μM) while it had negligible effect on the binding of [^{3}H]bradykinin, a selective B2 ligand. These findings suggest that, in the canine mesenteric artery, ovokinin appears to mediate vasorelaxation through the B1 receptor (EC_{50}=6.3 μM). However, ovokinin is much less potent than bradykinin: the EC_{50} value of the former is three orders of magnitude larger than that of the latter (Fujita, Nakagiri, and Yoshikawa, 1995[b]).

Because ovokinin also shares 4 common residues with angiotensin II, Fujita, Nakagiri, and Yoshikawa (1995[b]) investigated whether it also binds to either of the angiotensin II receptor subtypes (AT_1 and AT_2). It has been shown that another peptide that interacts with the B1 receptor, the B1 antagonist, des-Arg^9-[Leu^8]bradykinin, binds with relatively high affinity at both the AT_1 and AT_2 receptors (Pruneau et al., 1995). AT_1 receptors appear to be responsible for the vasoconstriction by angiotensin II (Pruneau et al., 1995). Fujita, Nakagiri, and Yoshikawa (1995[b]) utilized rat liver membranes and a selective AT_1 receptor ligand, [^{125}I]-Sar^1-Ile^8-angiotensin II in binding assays. Ovokinin inhibited binding of the ligand. It was also shown in isolated endothelium-denuded guinea

pig aorta that, when pretreated with 10^{-4} M ovokinin, the contraction by angiotensin II was diminished. The authors concluded from this data that ovokinin acts as a weak AT_1 antagonist.

Ovokinin has also been shown to bind to AT_2 receptors (Ki = 85 μM) with higher affinity than to AT_1 receptors (Ki = 569 μM) (Yoshikawa et al., 1997). The minimum sequence required for AT_2 receptor binding was RADHPF and was named ovokinin III. This fragment had no affinity for AT_1 receptors and very little for B1 receptors. Studies in normotensive Wistar rats in vivo indicate that ovokinin binds to AT_2 receptors which mediate antihypertensive activity. When administered to these rats intravenously, ovokinin causes a decrease in blood pressure which was blocked by the AT_2 receptor antagonist, PD123319.

2) Endothelium Dependence

It has been shown that the actions of many bioactive peptides are dependent upon the endothelium of blood vessels. The binding of such peptides to receptors on the endothelium stimulates the release of mediators, such as nitric oxide (NO) and prostaglandins, which affect the adjacent vascular smooth muscle to either relax or contract. Relaxation by ovokinin of the canine mesenteric artery was determined to be partially endothelium dependent (Fujita et al., 1995[a]). In endothelium-denuded preparations, ovokinin acted to relax the arteries (35% by 10 μM ovokinin), but to a smaller magnitude than in endothelium-intact samples (68% by 10 μM ovokinin). Ovokinin appears to bind to B1 receptors on the vascular smooth muscle as well since the peptide's effect was completely blocked by the B1 antagonist in the denuded arteries.

3) Effects of Various Inhibitors

It has been suggested that the vasodilatation produced by bradykinin may be mediated through nitric oxide and prostaglandins (Regoli et al., 1996). Fujita et al.

(1995[a]) made use of several inhibitors in an attempt to understand the mechanism of action for ovokinin. Indomethacin and aspirin, which are cyclooxygenase inhibitors, completely blocked the relaxation by ovokinin, whereas the inhibitors of NO synthase, NAME and NMMA, did not. This was true for both endothelium-intact and denuded arteries, indicating that, once bound, B1 receptors on both the endothelium and the vascular smooth muscle may stimulate the production of arachidonic metabolites. In fact, results from radioimmunoassays performed to measure prostaglandin I_2 and E_2 support this idea. Addition of 10 μM ovokinin induced only small increases in PGE_2 in arteries with or without endothelium. However, a large increase in PGI_2 was released from endothelium intact samples. Results from denuded arteries indicate that a small but significant amount is released from the vascular smooth muscle, however it appears that the majority of increased PGI_2 released is from the endothelium. In addition, the notion that nitric oxide does not play a role in this case is verified by the use of cAMP and cGMP phosphodiesterase inhibitors. Nitric oxide is known to stimulate soluble guanylate cyclase whereas PGI_2 stimulates adenylate cyclase. Indeed, the cAMP phosphodiesterase inhibitors augmented the relaxation by ovokinin but the cGMP phosphodiesterase inhibitor had no effect.

Inhibitors of phospholipase A_2, an enzyme which releases arachidonic acid from the membrane, were also utilized. These resulted in a small but significant inhibition of the relaxation produced by ovokinin (10μM). It has been demonstrated that both B1 and B2 receptors can activate phospholipase C (Marceau et al., 1997). However, a phospholipase C inhibitor did not have any effect on the vasorelaxation in the canine mesenteric artery.

TABLE 1: Effects of inhibitors on relaxation by ovokinin (10μM).

Inhibitors	Effect	Enzyme Inhibited
Aspirin Indomethacin	X	Cyclooxygenase
NAME NMMA	0	NO Synthase

Manoalide Quinacrine	↓	Phospholipase A_2
Gro-PIP	0	Phospholipase C
Rolipram IBMX	↑↑	cAMP Phosphodiesterase
Zaprinast	0	cGMP Phosphodiesterase

0 = No effect; ↓ = small but significant inhibition; X = complete inhibition; ↑↑ = significant augmentation.

c. In vivo Studies

The effects of ovokinin in vivo were observed in normotensive Wistar and spontaneously hypertensive rats. As mentioned above, ovokinin was administered intravenously to the normotensive rats at a dose of 5 mg/kg which resulted in a decrease in systolic blood pressure of 10 mmHg. Blood pressure was measured directly by carotid artery catheterization (Yoshikawa et al., 1997). In the spontaneously hypertensive rats, the peptide was administered orally as either a solution or an emulsion in 30% egg yolk. Systolic blood pressure differences were measured by the tail cuff method. A maximum of a 14.5 mmHg decrease was seen 2 hours after the administration of a 100 mg/kg ovokinin solution, however a solution of 25 mg/kg was ineffective. The antihypertensive activity was potentiated by the emulsification of the peptide in egg yolk. The emulsion was effective even at 25 mg/kg resulting in a maximum decrease of 17.6 mmHg after 4 hours. At the higher dose (100 mg/kg), the antihypertensive activity lasted beyond 8 hours with a maximum decrease of 25.0 mmHg after 4 hours. Explanations offered by Fujita, Sasaki, and Yoshikawa (1995[c]) for the potentiation of effect by emulsification were improved intestinal absorption and possible protection of the ovokinin from peptidases.

2. Species- and Tissue-Dependent Effects of Bradykinin

Bradykinin receptor classification and mechanisms of action remain somewhat unclear despite the active research being conducted in this area. The development of selective B1 and B2 receptor antagonists has been helpful in trying to fully understand the subtypes, however it remains complex because bradykinin receptor populations and the pathways with which they are linked vary among tissues and species (Regoli and Barabe, 1980; Scholkens, 1996; Feletou et al., 1994; Cherry et al., 1982). Even within the same species, such as the rabbit, differences in the effects of bradykinin are seen. Forstermann, Hertting, and Neufang (1986) found that the relaxation by bradykinin in the extrapulmonary artery was much smaller in magnitude than in the coeliac or mesenteric arteries of the rabbit.

In addition, the rates of kinin production and degradation, which also vary widely, will partly determine the extent of the action by bradykinin in a particular animal and tissue (Regoli and Barabe, 1980; Mombouli and Vanhoutte, 1995). It has been documented that some tissues, such as guinea pig ileum, have very active kininases which results in the reduction of either the magnitude or duration of kinin activity. This can be shown with the use of inhibitors of ACE or other kininases. However, other isolated smooth muscle preparations react to kinin with a stable contraction or relaxation where the enzyme, although present, does not interfere with the activity (Regoli and Barabe, 1980). Generally, it appears that, typically in blood vessels, the major B1 effect is contraction while that of B2 is vasorelaxation (Wiemer and Wirth, 1992).

Subtyping of the bradykinin receptors is based mostly on the order of potency of agonists: (Regoli et al., 1996)

B1: LysdesArg^9-bradykinin > desArg^9-bradykinin > bradykinin
B2: bradykinin > LysdesArg^9-bradykinin > desArg^9-bradykinin

However, further subtyping of the B1 and B2 receptors has been suggested since there appear to be differences in agonist and antagonist binding across species and tissue types (Regoli et al., 1992; Regoli et al., 1996 for review). Certain species and tissues are believed to be monoreceptor preparations, such as the rabbit aorta (Regoli et al., 1980; Regoli et al.,

1996), while others, like the canine renal artery, express both the B1 and B2 subtype receptors (Rhaleb et al., 1989). B2 sites have been localized on both the endothelium and smooth muscle (canine renal artery) or on the endothelium only (canine carotid artery) (Regoli et al., 1996). Interestingly, B1 receptors appear to be up-regulated following certain types of tissue injury (Regoli et al., 1980; Marceau, 1995), including lipopolysaccaride (LPS) treatment. In studies of certain isolated tissues, where injury to the vessel during preparation and incubation most likely occurs, it has been demonstrated that response to the B1 agonist, desArg^{9}-bradykinin, increases as a function of time.

Recently, the cloning and expression of both B1 and B2 subtype receptors from several species were achieved. This information indicated that both receptor subtypes are coupled to G proteins and have seven transmembrane domains. The genes for the receptors have been cloned in several species, including the human (Menke et al., 1994; Hess et al., 1992), mouse (McIntryre et al., 1993; Ma et al., 1994), and rat (McEachern et al., 1991). In the human, it was found that the B1 receptor had only 36% overall sequence homology with the B2 receptor. There are also species related differences in the predicted sequences of the B2 receptor: the human B2 receptor is approximately 80% homologous with the mouse and rat B2 receptor. B2 receptors are widely expressed normally while B1 receptors have been shown to be absent from most normal tissues and species, with certain exceptions, such as the dog. In particular, in all cultured endothelial cell types studied to date, B2 receptors have been demonstrated pharmacologically while only some types indicate B1 receptor activity (Wohlfart et al., 1997; Sung et al., 1988). A study on the detection of B1 and B2 receptor mRNA using PCR techniques has recently been conducted and shows that B2 mRNA can be demonstrated in bovine aortic, rat microvascular coronary, and human umbilical vein endothelial cells whereas B1 receptor mRNA is only identified in the latter two types (Wohlfart et al., 1997)

It has been suggested that both B1 and B2 receptors can interact with different G proteins coupled to various second messenger pathways (Regoli et al., 1996) (Figure 2). Phospholipase C (PLC) can be activated (Clark et al., 1986) which will lead to increased phosphatidylinositol turnover resulting in inositol triphosphate production (O'Flaherty, 1987 for review; Derian and Moskowitz, 1986). The inositol triphosphate diffuses to the

endoplasmic reticulum and binds to receptors to release Ca^{2+} stores resulting in an increase in $[Ca^{2+}]_i$ (Morgan-Boyd et al., 1987). In addition, the transient increase of cytosolic Ca^{2+} levels was found to be partially dependent on extracellular Ca^{2+} as well as intracellular stores (Morgan-Boyd et al., 1987). This mediates the constriction seen in certain arteries (rabbit aorta) and veins (rabbit jugular vein). The increased intracellular Ca^{2+} levels can also lead to the release by Ca^{2+}-dependent enzymes of second messengers, such as nitric oxide (Blatter et al., 1995; Cocks et al., 1985) and prostaglandins. NO diffuses from the endothelium to the smooth muscle where it activates guanylate cyclase and results in relaxation due to increased levels of cGMP (Wiemer and Wirth, 1992, Wiemer et al., 1991; Boulanger et al., 1990). Diacylglycerol, which is also produced by the action of PLC on phosphatidylinositol diphosphate, activates protein kinase C by forming a membrane-associated complex (O'Flaherty, 1987). In addition, the receptors may be coupled to phospholipase A_2 which will free arachidonic acid from membrane phospholipids and lead to the production of prostanoids (Burch and Axelrod, 1987; Regoli et al., 1996; Rhaleb et al., 1989; Forstermann, Hertting, and Neufang, 1986).

Although both receptor subtypes can be coupled to the same second messenger pathways, it has been demostrated that some differences do exist. For example, in cultured rabbit superior mesenteric smooth muscle cells, the BK induced inositol phosphate formation is less dependent on extracellular Ca^{2+} levels than the effect by desArg^9-BK (Tropea et al., 1993). There are also differences in the maximum responses elicited by the B1 agonist, des-Arg^9-bradykinin and the B2 agonist, bradykinin, and the concentrations needed to produce that maximum response. In cultured endothelial cells, des-Arg^9-BK produced an increase in $[Ca^{2+}]_i$ but required a 100-fold higher concentration to achieve a maximum response which was considerably lower than that of bradykinin (Sung et al., 1988). Similar results were seen with cultured bovine pulmonary artery endothelial cells (Morgan-Boyd et al., 1987) and bovine aortic endothelial cells (D'Orleans-Juste, de Nucci, and Vane, 1989). In the latter and in rat microvascular coronary endothelial cells, higher concentrations of des-Arg^9-BK compared to bradykinin were also needed for maximum increases in intracellular cGMP levels (Wohlfart et al., 1997).

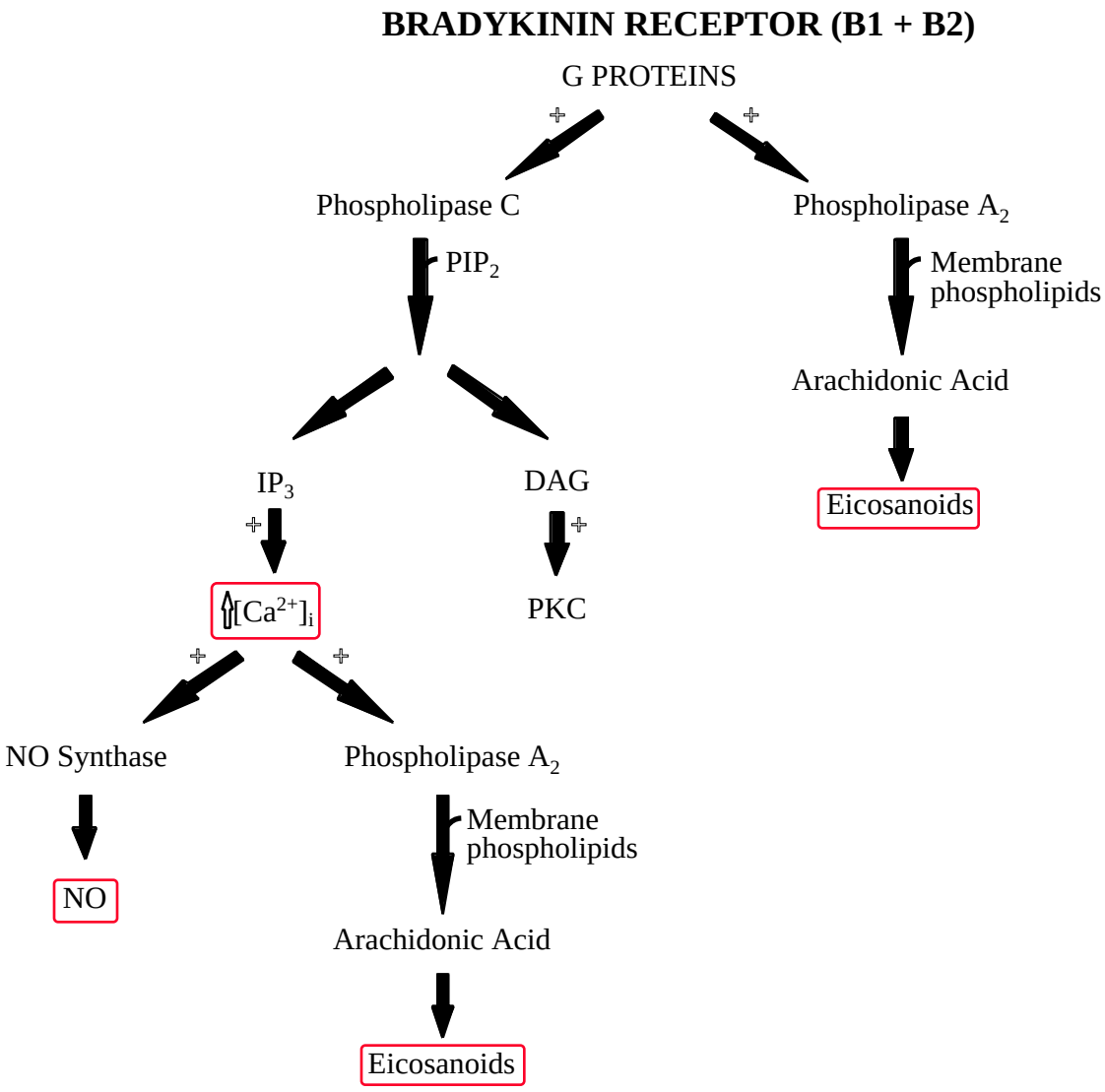

FIGURE 2: Second messenger pathways coupled to bradykinin receptors. PIP_2 = phosphatidylinositol biphosphate; IP_3 = inositol triphosphate; DAG = diacylglycerol; PKC = protein kinase C; NO = nitric oxide. Adapted from Regoli et al., (1996).

The effects of bradykinin have been demonstrated to be of short duration. Blatter et al. (1995) have shown that bradykinin induces a rapid and transient increase in $[Ca^{2+}]_i$ that reached a maximum concentration over 10 ± 1s and returned to basal levels after 350 ± 20 s. The production of NO was longer lasting and was first detected approximately 5 seconds after the Ca^{2+} release. This transient effect has also been demonstrated in vitro in cultured porcine aortic endothelial cells. It was shown that bradykinin stimulated the release of arachidonic acid, but the response was transient, with the maximum effect occurring between 2 and 4 minutes (Whorton et al., 1982). In guinea pig aortic smooth muscle cells, the rapid increase in $[Ca^{2+}]_i$ has been shown to be mediated by the B2 receptor. It occurs within 20 seconds and returns to basal levels after approximately 150 seconds. The source of the calcium seems to be from intracellular stores and a Ca^{2+} influx.

3. Degradation of bradykinin

Bradykinin injected in vivo has a very short half-life which demonstrates the body's high capacity for degradation. This assures the prevention of unwanted effects in non-target areas. Bradykinin can be degraded by both exo- and endopeptidases. Hydrolysis of any of the bonds in the peptide renders it unable to bind and activate the B2 receptor (Skidgel, 1992). Kininase II, which is identical to angiotensin converting enzyme (ACE), kininase I, otherwise known as carboxypeptidase N, and neutral endopeptidase 24.11 (NEP) are involved in the degradation process (Erdos and Skidgel, 1987). Kininase I, located in the plasma, cleaves the C-terminal arginine producing des-Arg^9-bradykinin which, as mentioned above, binds with high affinity to the B1 receptor. Both ACE and NEP act at the same site and remove Phe-Arg from the C terminus and are anchored to the plasma membrane which allows them to act on bradykinin near the peptide's receptors. ACE is also found in the plasma, which most likely originates from the membrane-bound enzyme on endothelial cells (Costerousse et al., 1992), and is possibly present on non-endothelial areas of the vascular wall (Rouissi et al., 1990). Other bradykinin degrading enzymes seem to be located intracellularly. In human umbilical vein endothelial cells, it

was demonstrated that the endothelial kininase activity increased after homogenization of the cells (Graf et al., 1992).

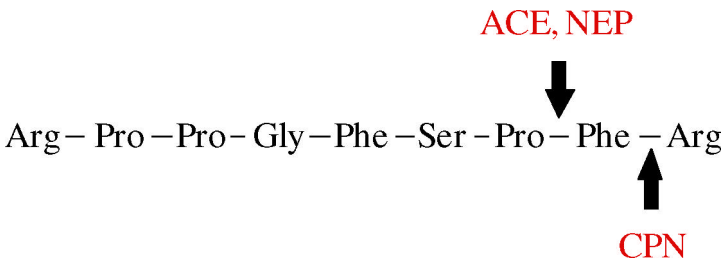

FIGURE 3: Sites of action of bradykinin degrading enzymes. ACE = angiotensin converting enzyme (kininase II); NEP = neutral endopeptidase 24.11; CPN = carboxypeptidase N (kininase I).

ACE appears to be the most important enzyme involved in the metabolism of bradykinin (Rouissi et al., 1990). For example, in human umbilical vein endothelial cells, ACE and NEP are the main kininases involved in the degradation of bradykinin with ACE activity being four-fold higher than NEP (Graf et al., 1992). ACE was also found to be responsible for most of the bradykinin degradation in rat coronary circulation (Ersahin and Simmons, 1997). The enzyme possesses two functional active sites, both of which hydrolyze bradykinin well. The sites function independently and exhibit some differences, including the degree of chloride dependency and sensitivity to ACE inhibitors, which suggests structural differences between these two sites (Costerousse et al., 1992). ACE inhibitors interact with these active sites and are characterized as slow and tight binding competitive inhibitors (Unger and Gohlke, 1994).

Hecker, Dambacher, and Busse(1992) have demonstrated with radioimmunoassay that, in the presence of ACE inhibitors, there is accumulation of bradykinin near the endothelial cells, which in turn, will influence the amounts of the second messengers, NO and prostacyclin, produced by the cells in response to bradykinin (Wiemer et al., 1991).

ACE inhibitor, there is an increase in the resting intracellular Ca^{2+} levels as well as an enhancement of the magnitude of the Ca^{2+} increase induced by bradykinin (Hecker, Dambacher, and Busse, 1992).

4. Spontaneously Hypertensive Rats

It has been shown that, during the course of hypertension and aging, the endothelium undergoes changes both functionally and morphologically (Falloon et al., 1993). Falloon et al. (1993) found that thickness of the media of mesenteric resistance arteries from Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR) was significantly greater in the latter (18.6 ± 0.6 and 25.9 ± 1.4 μm, respectively) and lumen diameter was larger in WKY (302.7 ± 3.9 and 282.7 ± 5.8 μm, respectively). It logically follows that responses that are endothelium-dependent may also be altered. For example, the maximal relaxation to acetylcholine at high concentrations (10^{-6} - 10^{-5} M) was decreased with advancing age in both spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto rats. This decreased effect was demonstrated earlier in SHR (3-6 months) than in normotensive rats (12-25 months) (Koga et al., 1989). The release of the endothelium-derived relaxing factor (EDRF) does not seem to be altered, but instead, this effect seems to be due to simultaneous release of an endothelium-dependent contracting factor (Fu-Xiang et al., 1992). There are differing reports concerning the identity of the endothelium-derived contracting factor (EDCF), but studies seem to indicate that it is a product of the cyclooxygenase pathway (Koga, 1989; Luscher and Vanhoutte, 1986). In the mesenteric vascular bed of SHR preconstricted with norepinephrine, the endothelium-dependent relaxations by bradykinin have also been shown to be impaired, even at a young age (7 weeks) (Wirth et al., 1996).

Observations have been made concerning contracting factors as well, however the data have not been consistent. Some studies found that certain vessels, such as the aorta, renal artery and mesenteric artery, of SHR and Wistar-Kyoto rats have similar contractions to norepinephrine (Konishi and Su, 1983; Fu-Xiang et al., 1992). Others have shown that

contractions by norepinephrine are smaller in magnitude in normotensive Wistar versus SHR aortas (Wirth et al., 1996). Still others have found that SHR aortic rings contract less than normotensive samples to 10^{-4} M norepinephrine (2.8 ± 0.8g and 4.0 ± 1.3 g, respectively) but are also more responsive to lower concentrations of prostaglandin $F_{2\alpha}$ (10^{-8} - 10^{-7} M) (Luscher and Vanhoutte, 1986). This discrepancy in results may be explained partly by the differing age of the rats used in these studies, which ranged from 4 weeks to 51 weeks. Wirth et al. (1996) found that contractions by norepinephrine in both normotensive rat and SHR aortas increased in magnitude as age increases.

CHAPTER II

MATERIALS AND METHODS

A. Experimental Protocol

Male Hartley guinea pigs, Sprague Dawley rats, and spontaneously hypertensive rats were purchased from Charles River Laboratories and were approximately 6 to 7 weeks, 18 to 21 weeks, and 14 weeks, respectively, at time of experimentation. The animals were allowed to acclimate for at least one week before the aortas were harvested for use in the isolated tissue studies. All animals were housed in the animal facilities at Virginia-Maryland Regional College of Veterinary Medicine with a light cycle of 10 hours light/ 14 hours dark and allowed free choice access to standard rat chow and water. Animal procedures in this study were approved by the Virginia Tech Animal Care Committee.

The animals were injected with heparin IP (3000 units in guinea pigs, 2000 units in rats) approximately 30 minutes prior to euthanasia in order to avoid blood clotting and possible damage to the endothelium of the aorta (Fulton, McGiff, and Quilley, 1996). The animals were then placed in a small, closed cage and gassed with CO_2 until euthanized. The process lasted between 3 and 6 minutes. The aortas were then removed and cut into rings approximately 3.5 mm wide. Only the first four segments distal to the aortic arch were used (Figure 4), due to apparent differences in magnitude of contraction resulting from aortic rings past the 4th segment. Regional variation in reactivity was also seen in thoracic aortas of guinea pigs in a study by Gregg et al. (1995).

The rings were mounted between two stainless steel hooks in individual 20 ml tissue baths containing Krebs-Henseleit solution (120 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$, and 10 mM glucose) at 37^{o}C, pH 7.4, and bubbled with 95% O_2/ 5% CO_2 (Fujita et al., 1995[a]). One hook was

fixed in position while the other was attached to the lever of a force-displacement transducer (Figure 5). Tissue contractile force was amplified using the manifold Transbridge TBM4 by World Precision Instruments and converted to digital form using the MacLab system and software.

A resting tension of 2 g applied to the rings was found to produce maximal results by testing 3 different tensions and observing their effects. Calibration of the system was verified before and after each assay. The preparation was allowed to equilibrate for 2 hours, during which the solution in the baths were exchanged every 15-20 minutes. The aortas were then precontracted with one of four agents: bradykinin, phenylephrine, prostaglandin $F_{2\alpha}$, or KCl. Concentrations of these agents eliciting maximum contraction were used as determined by cumulative dose-response curves conducted in guinea pig and Sprague-Dawley rat aortic rings. A typical dose-response curve is shown in Figure 6. No dose-response studies were performed on spontaneously hypertensive rat aortas due to the limited number of available animals, therefore concentrations for normotensive rats were used. Clineschmidt et al. (1970) found that the dose which achieved the maximum response in normotensive rats was similar to that in spontaneously hypertensive rats.

TABLE 2: Concentrations of Contracting Agents Used For All Animals

Contracting Agent	Concentration Used
Bradykinin	10^{-5} M
Phenylephrine	1.5×10^{-4} M
Prostaglandin F_2	10^{-5} M
Potassium Chloride	90 mM

Once a plateau in the contraction had been reached, either ovokinin (10^{-4} M) or an equivalent volume of saline (control) was added to the bath. In the inhibitor studies, captopril (5×10^{-6} M) or saline (control) was incubated with guinea pig aortic rings for approximately 30 minutes. The rings were then contracted with bradykinin, and ovokinin

was added to both the inhibitor-treated and control rings at the plateau of the bradykinin contraction.

At the end of the study, acetylcholine (10^{-6} M) was used to verify an intact endothelium in the rings from both strains of rats. However, acetylcholine did not induce significant relaxations in the isolated guinea pig aorta preparations. Similar observations have been seen by other groups and may involve decreased levels of cGMP being produced in the smooth muscle in response to acetylcholine relative to other endothelium dependent vasoactive substances such as substance P (Hozumi, Fukuta, and Suzuki, 1997). It has also been shown that the vasorelaxation by acetylcholine in the guinea pig thoracic aorta varies depending on the region of the aorta being studied. Isolated proximal segments relaxed very little to ACh, with the response increasing distally to the level of the diaphragm (Gregg et al., 1995). The calcium ionophore, A23187, does not exhibit this regional variation and induces endothelium dependent relaxations as well (Peach, Singer, and Loeb, 1985). In addition, Furchgott (1983) reports that A23187 is 10-30 times more potent than ACh and more powerful against high levels of contraction. Therefore, A23187 (10^{-7} M) was used to physiologically verify the presence of endothelium in guinea pig aortic rings.

Bradykinin, prostaglandin $F_{2\alpha}$, phenylephrine, captopril, calcium ionophore A23187 (free acid), and acetylcholine were puchased from Sigma Chemical Company. Ovokinin was custom synthesized at Peptides International (Louisville, Kentucky),with a purity of 95% or greater. All solutions were freshly made prior to experimentation. A23187 was dissolved in DMSO and the amount used in the assay was less than 1% of the total volume of the bath. DMSO alone had no effect on the aortic ring.

Mass spectral analysis on a frozen stock solution of ovokinin prepared approximately one month prior and stored at -20^{o} C was performed to verify that there was no alteration in the peptide during that period. Indeed, the mass spectrum of the ovokinin in solution was very similar to that provided by the vendor, thus suggesting that the peptide had not been altered by storage (Figure 7).

B. Statistical Analyses

All results are presented as means ± SE. The time at which ovokinin was added to the bath is considered t = 0, and contraction in the ring at this time is considered the maximum contraction. Readings of contraction were taken every 30 seconds and were expressed as a percent of the maximum. Three-way ANOVAs (animal x treatment x time) were conducted for the first three minutes. Student's *t* test for unpaired comparisons was performed on the data comparing the contractions by the four agents in normotensive versus spontaneously hypertensive rats and comparing treatment and control data at individual time points. P values <0.05 were regarded as significant (Sokal and Rohlf, 1995).

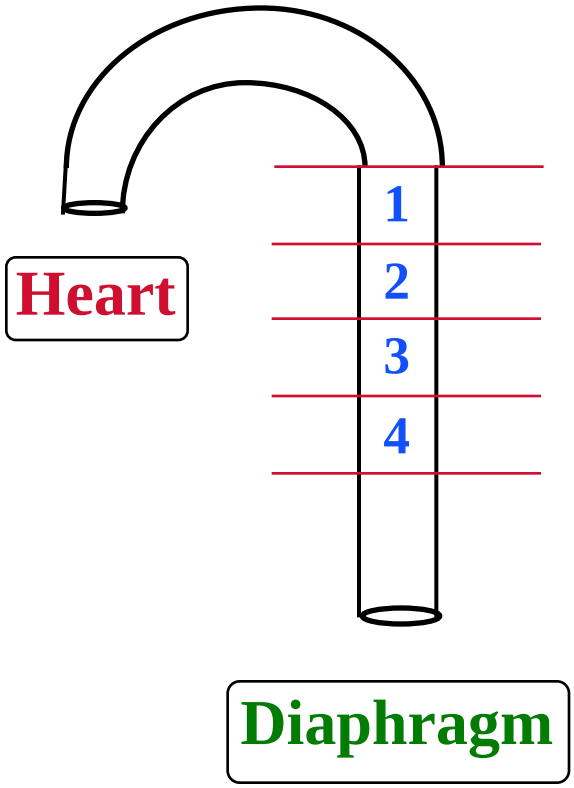

FIGURE 4: A schematic of the aorta. The first cut was made immediately after the bend of the aortic arch. Segments 1-4, approximately 3.5 mm wide, were used in this study.

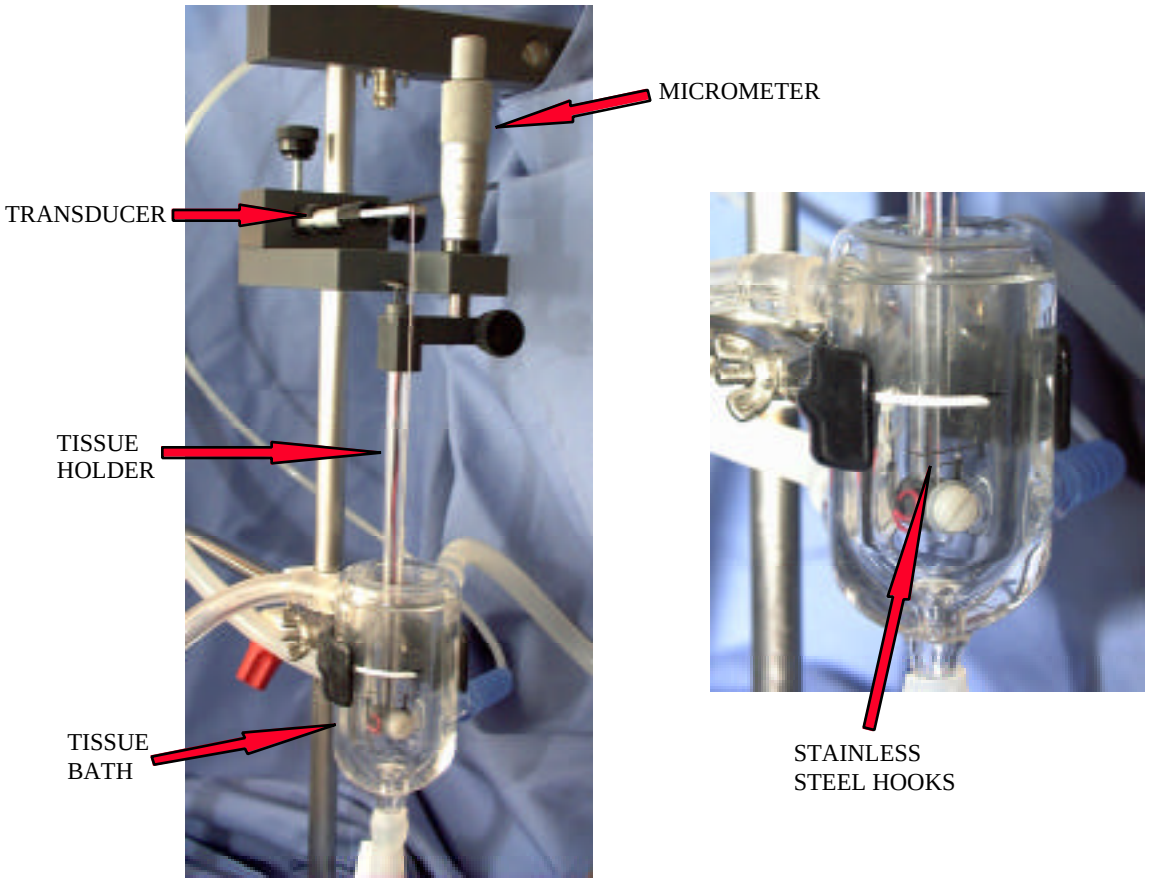

FIGURE 5: Isolated tissue bath apparatus

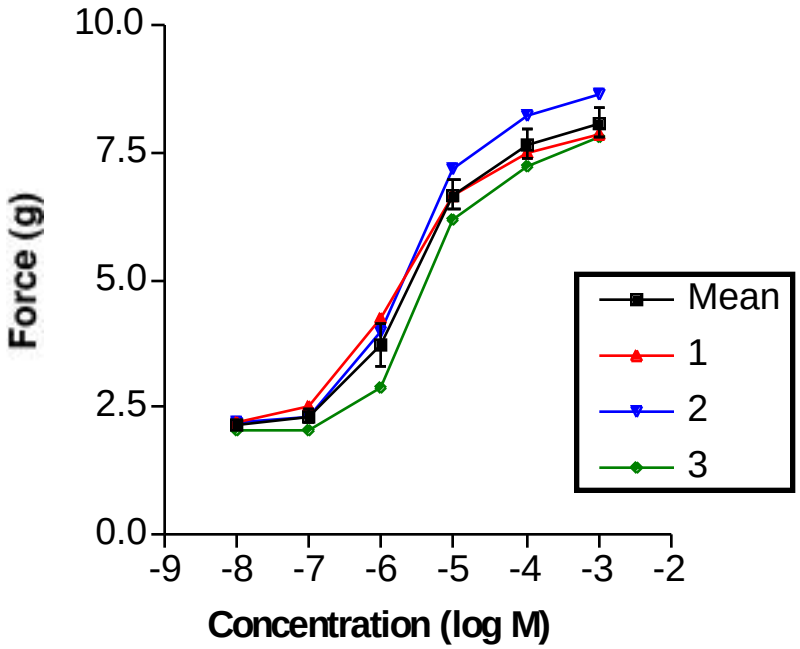

FIGURE 6: Example cumulative dose-response curve for phenylephrine in the guinea pig. Results from 3 aortic rings (1, 2, 3) and the mean are shown.

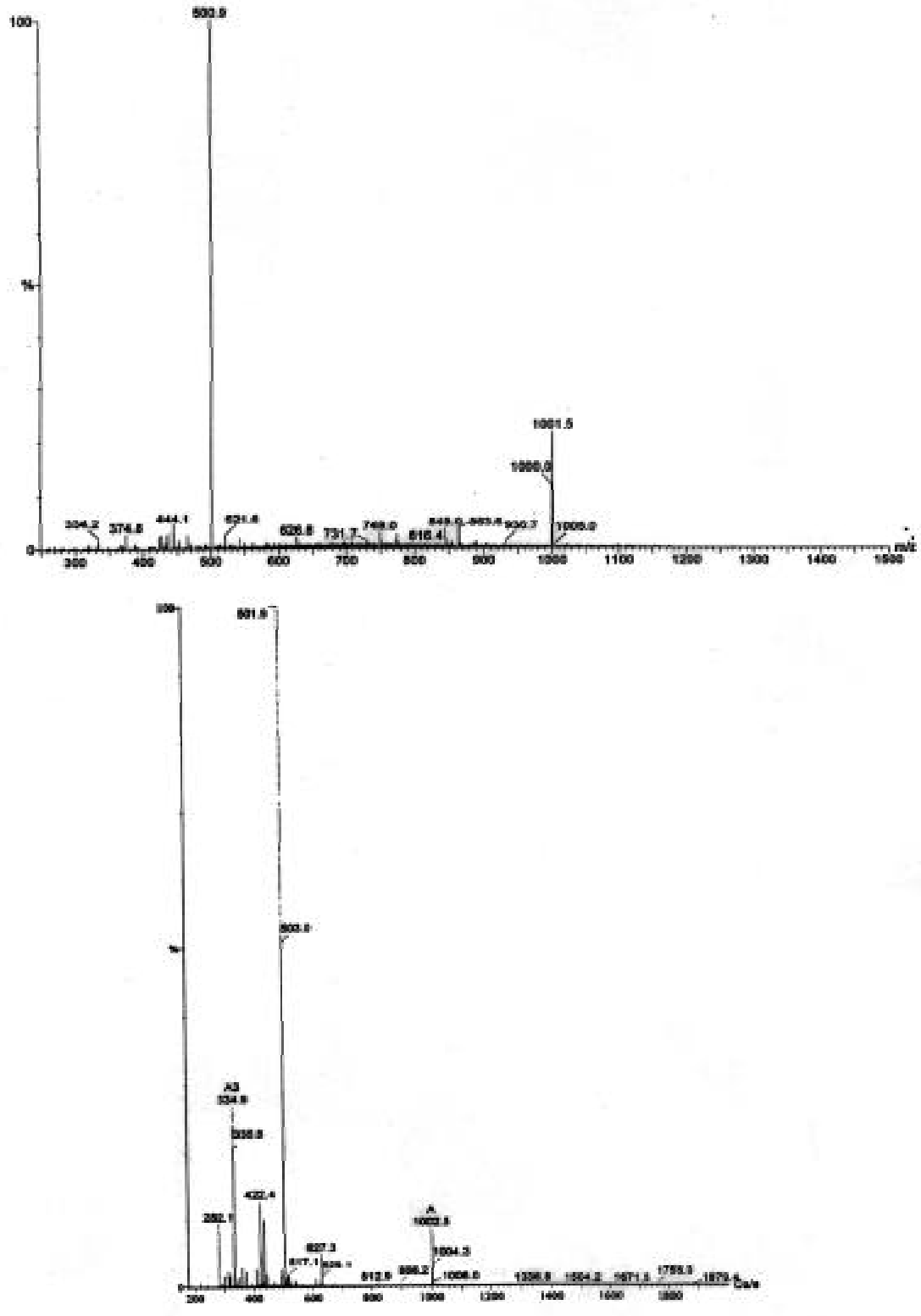

FIGURE 7: Mass spectral analysis performed on frozen stock solution of ovokinin stored at -20° C for approximately 1 month (top) and provided by vendor (bottom).

CHAPTER III

RESULTS

A. Isolated Guinea Pig Aorta

The addition of phenylephrine, prostaglandin $F_{2\alpha}$, and KCl to the bath elicited strong and sustained contraction in isolated guinea pig aorta. Representative contractions by all four agents are shown in Figure 8. Effects of phenylephrine consisted of a rapid contraction followed by a slower development of tension until the maximum contraction was reached. This observation was also seen in a study on isolated guinea pig aorta by Molderings and Schumann (1989). Contractions by prostaglandin $F_{2\alpha}$ and KCl generally developed more slowly and required a longer period of time to reach a plateau. In this preparation, the bradykinin-induced contractions were smaller in magnitude than those by the other contracting agents, averaging 0.85 ± 0.08 grams, and were transient, returning to resting tension within 10 minutes.

Ovokinin did not have any observable effect of its own (data not shown) nor did it have a statistically significant effect on aortic rings precontracted with phenylephrine, prostaglandin $F_{2\alpha}$, or KCl (Figure 9). Both control and ovokinin-treated vessels remained at approximately the same level of tension that was present at time zero. However, ovokinin induced a potentiation of the contraction by bradykinin, with a maximum response occurring at 2 minutes. At this time, the ovokinin treated ring was at 108.7 ± 4.67% of the maximum contraction while the control ring was declining towards baseline at 96.7 ± 1.78% ($p = 0.0377$). Following this maximum response, the ovokinin-treated ring relaxed towards baseline as well and, at 3 minutes, the effect of ovokinin was no longer statistically significant.

B. Isolated Normotensive and Spontaneously Hypertensive Rat Aorta

Bradykinin had no observable effect on the isolated normotensive rat aorta, 0.08 ± 0.024% (n = 5). Surprisingly, it induced a small but significant contraction in the aorta of spontaneously hypertensive rats (Figure 10). The mean contraction by bradykinin in the SHR aorta, 0.39 g ± 0.047 (n = 10), was significantly smaller than that seen in guinea pig aortas. The effect of bradykinin in the SHR and guinea pig aorta was similar in both instances, the contraction was transient. The maximum contractions induced by the other agents, phenylephrine, prostaglandin $F_{2\alpha}$, and KCl, were larger in magnitude in normotensive compared to spontaneously hypertensive rats (Figure 11).

Ovokinin had no significant effect on aortic rings from normotensive rats regardless of the agent used to precontract the vessel (Figure 12). Similarly, ovokinin had no observable action in rings from hypertensive rats precontracted with bradykinin, phenylephrine, and KCl (Figure 13). But, in prostaglandin $F_{2\alpha}$–precontracted SHR aortas, the level of contraction in the control ring continued to increase slowly from t = 0 (104.7% ± 0.40) while the ovokinin-treated ring remained at approximately the same level (99.25 ± 1.95%). The difference in the trends of the ovokinin-treated and control curves in Figure 13(B) was significant (p = 0.01), but the effect of ovokinin at the time point where the maximum response occurred, t = 2.5 minutes, was not statistically significant.

TABLE 3: Summary of the Effects of Ovokinin on the Contraction by Four Agents

Contracting Agent	Guinea Pig	Normotensive Rat	SHR
Phenylephrine	0	0	0
Prostaglandin $F_{2\alpha}$	0	0	↓ **
KCl	0	0	0
Bradykinin	↑ ***	0	0

0 = no effect; ↑ = potentiation; ↓ = inhibition. **p=0.01; ***p=0.0008

E. ACE Inhibitor Studies

In guinea pig aortic rings pretreated with captopril (an ACE inhibitor) an increase in magnitude of contraction by bradykinin was seen compared to the control, 1.43 g ± 0.19 and 0.80 g ± 0.11, respectively ($p = 0.001$, $n = 7$) (Figure 14). After incubation with either captopril or saline (control) for 30 minutes, the rings were contracted with bradykinin. At the plateau of contraction, ovokinin was added to the baths, which caused potentiation of the bradykinin contraction in the control ring. However, this effect of ovokinin was blocked in the captopril-treated rings ($p < 0.0001$), which immediately began declining towards baseline (Figures 15 and 16). As with the guinea pig data reported above, the maximum effect by ovokinin was seen at 2 minutes, when the mean contraction of the control aortas was 110.6 % ± 1.83 whereas that of the treated rings was 87.2% ± 1.88 ($p < 0.0001$, $n = 7$). After 2 minutes, the non-treated rings began to relax towards baseline as well, however, at 3 minutes, there remained a significant difference between the captopril-treated and control aortas ($p < 0.0001$) (Figure 17).

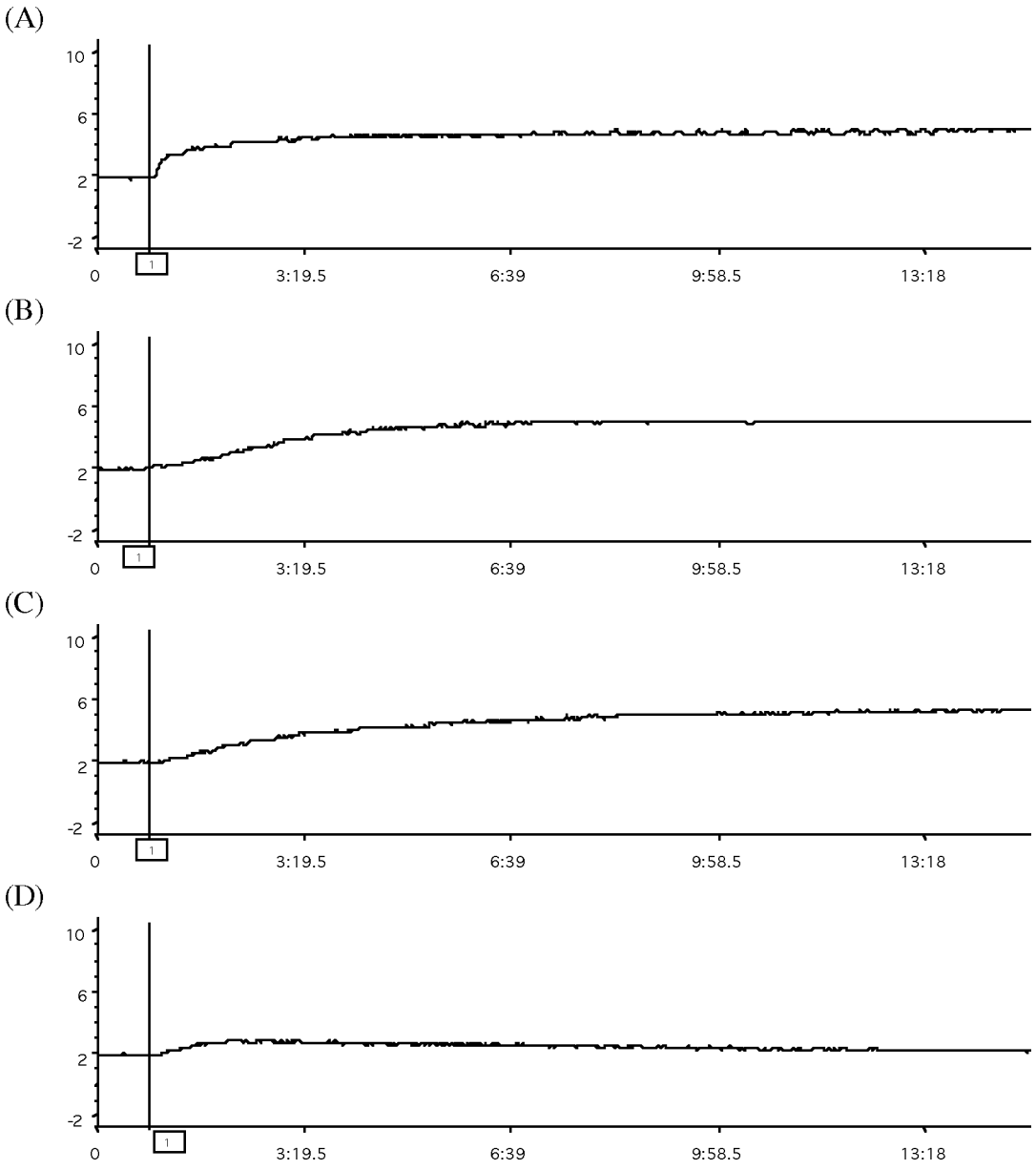

FIGURE 8: Representative tracings from isolated guinea pig aortic rings contracted by phenylephrine (A), prostaglandin $F_{2\alpha}$ (B), KCl (C), and bradykinin (D).

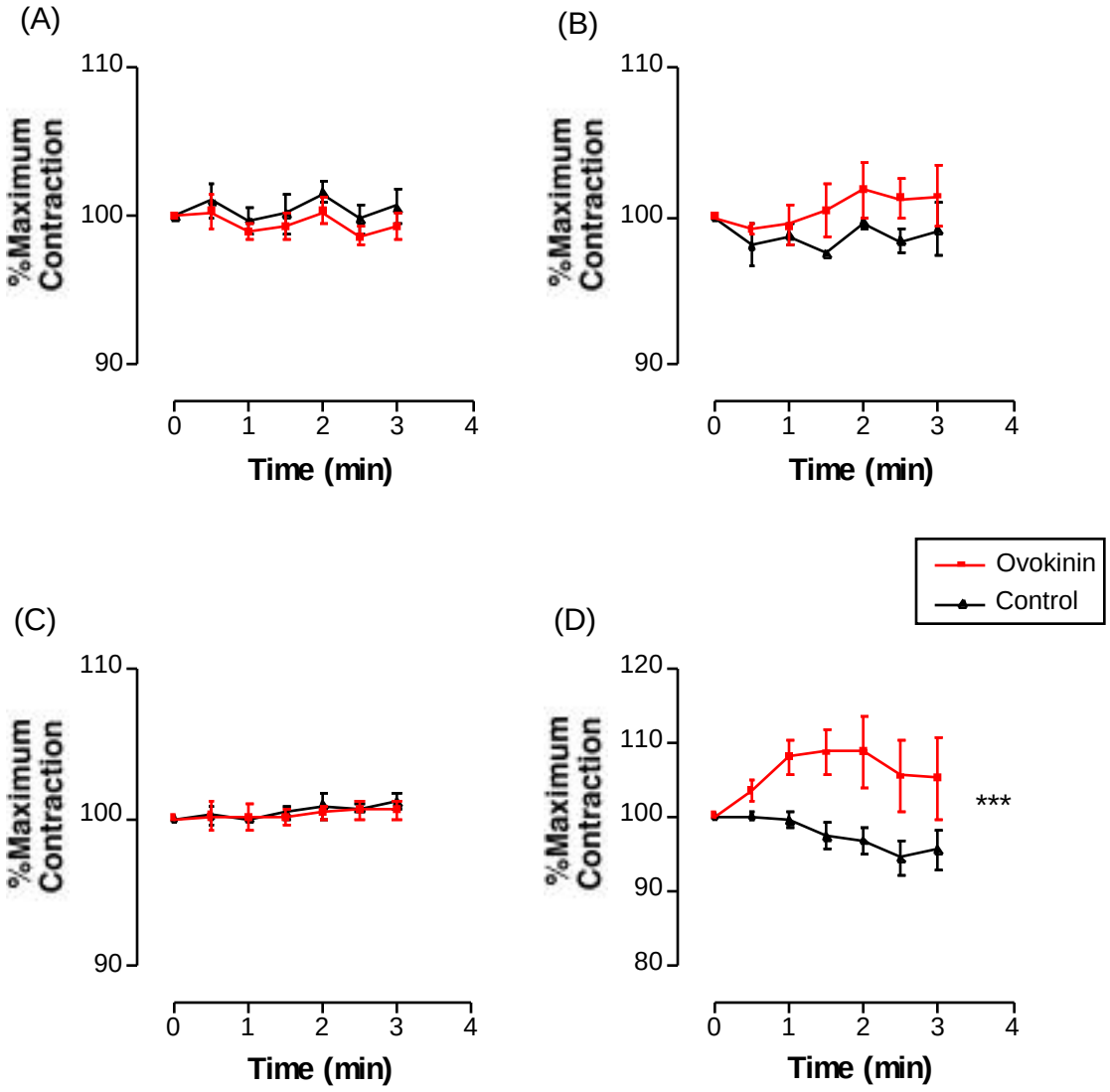

FIGURE 9: Effects of ovokinin on isolated guinea pig aorta precontracted with phenylephrine (A), prostaglandin $F_{2\alpha}$ (B), KCl (C), and bradykinin (D). ***$p < 0.001$, $n = 6$.

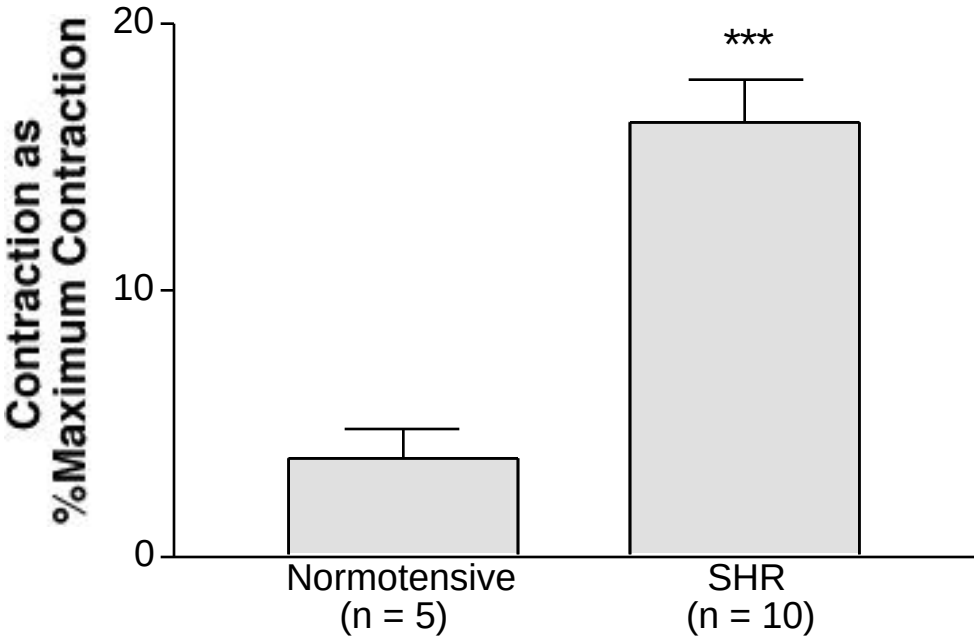

FIGURE 10: Differing effects of bradykinin on normotensive and SHR aortas. The amount of contraction is measured as a percent of the maximum contraction achieved. ***$p < 0.0005$.

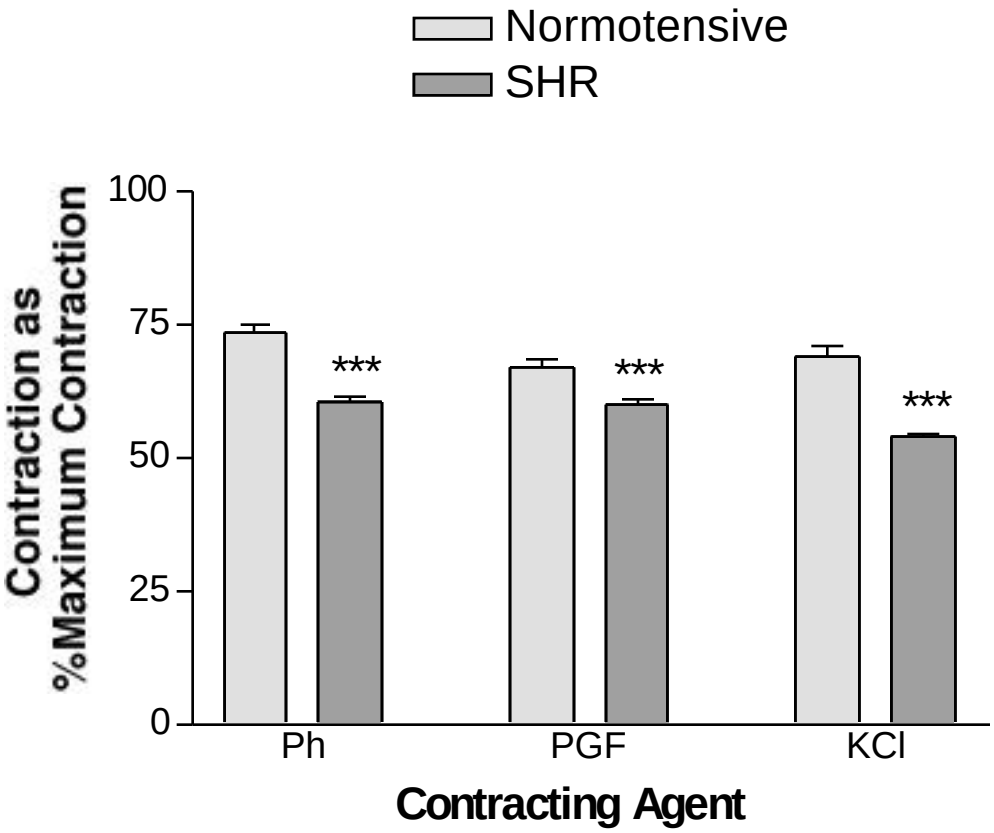

FIGURE 11: Comparison of contraction by phenylephrine (Ph), prostaglandin $F_{2\alpha}$ (PGF), and potassium chloride (KCl) in normotensive vs. spontaneously hypertensive (SHR) rats. ***$p < 0.001$ as compared to normotensive rats.

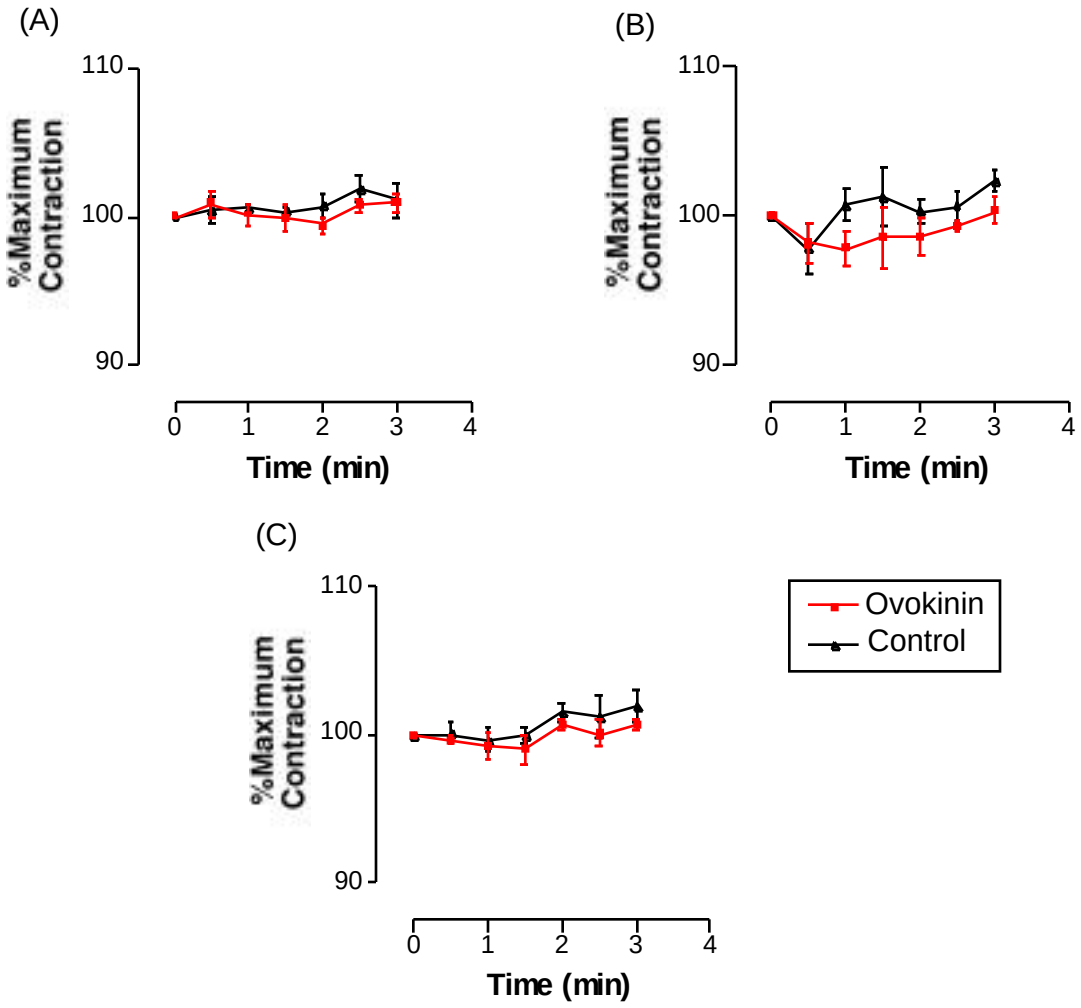

FIGURE 12: Effects of ovokinin on isolated normotensive rat aorta precontracted with phenylephrine (A), prostaglandin $F_{2\alpha}$ (B), and KCl (C). Results are not statistically significant.

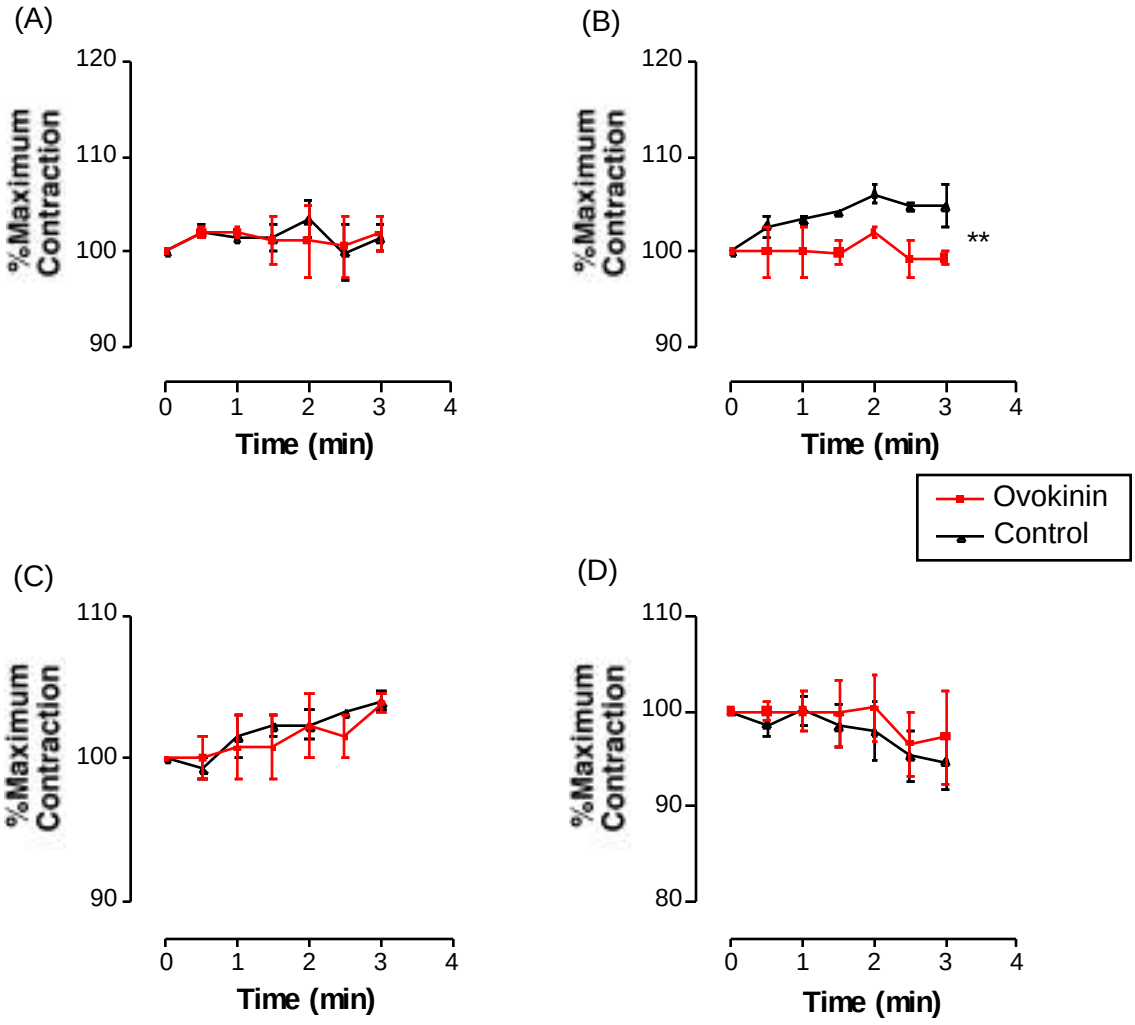

FIGURE 13: Effects of ovokinin on isolated spontaneously hypertensive rat aorta precontracted with phenylephrine (A), prostaglandin $F_{2\alpha}$ (B), KCl (C), and bradykinin (D). **p = 0.01

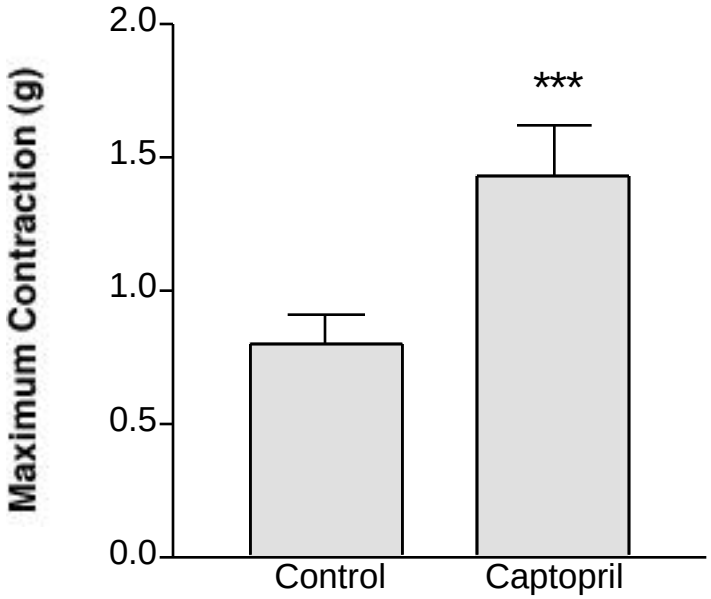

FIGURE 14: Effect of captopril pretreatment on the maximum contraction by bradykinin. ***p = 0.001, n = 7.

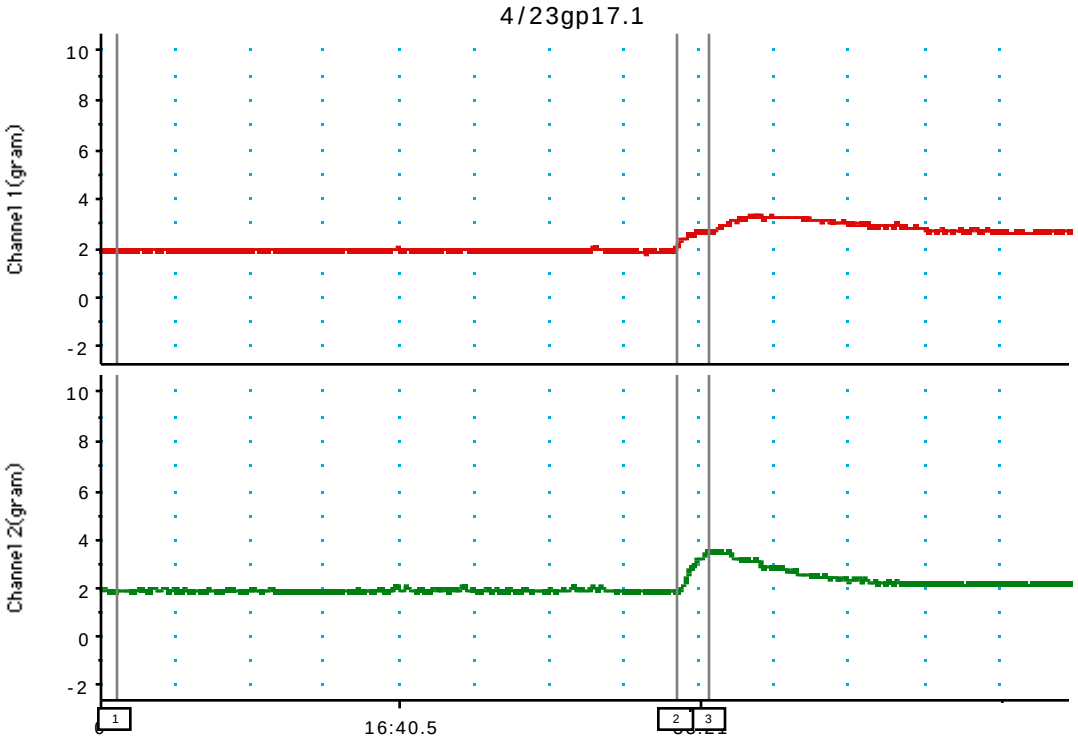

FIGURE 15: Typical tracing of aortic rings with or without captopril pretreatment from one guinea pig. [1] indicates saline (control) addition to Channel 1 and captopril (5×10^{-6} M) addition to Channel 2, [2] bradykinin (10^{-5} M) addition, and [3] ovokinin (10^{-4} M) addition.

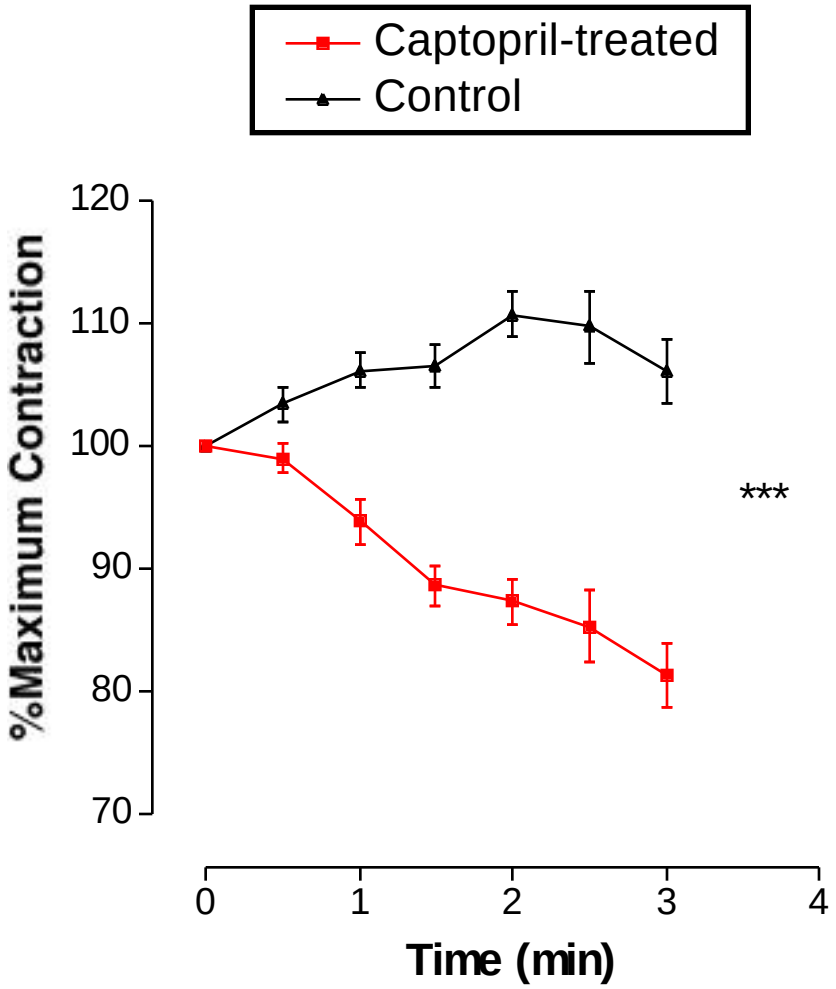

FIGURE 16: Effect of ovokinin on captopril-treated and control guinea pig aortic rings precontracted with bradykinin. ***$p < 0.0001$; $n = 7$.

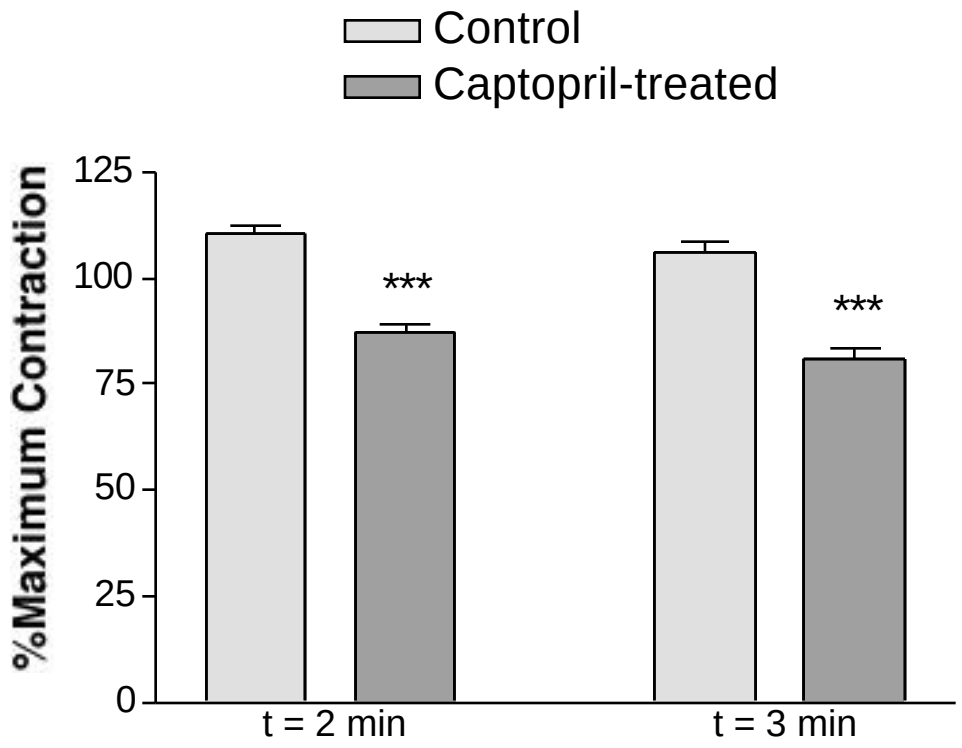

FIGURE 17: Percent of maximum contraction at 2 and 3 minutes after addition of ovokinin in saline (control) and captopril-treated aortic rings. *** $p<0.0001$ as compared to the control ($n = 7$).

CHAPTER IV

DISCUSSION

Bradykinin has been shown to have differing effects and mechanisms of action depending upon the tissue and species being studied. From the isolated tissue experiments conducted by Fujita et al. (1995[a]) and from the present study, it appears that ovokinin shares this characteristic with bradykinin. It has been suggested that, in the canine mesenteric artery, ovokinin acts through the B1 bradykinin receptor subtype to stimulate release of prostacyclin, thus inducing vasorelaxation. However, this does not seem to be the case with isolated guinea pig or rat aortas.

In this investigation, we found that bradykinin produced a significant contraction in guinea pig aortic rings (0.85 ± 0.08 grams). The effect was transient, returning to baseline within a short period of time. Regoli and Barabe (1980) offered suggestions as to why bradykinin induces contractions of such short duration, one of which is rapid degradation of the peptide due to high concentrations by active kininases present in the tissue. Rouissi et al. (1990) suggested that because evidence of bradykinin degradation is also seen in isolated vessel studies where low concentrations of the peptide are used (8×10^{-9} M), the affinity of ACE for bradykinin should equal, if not exceed, that of the bradykinin receptor sites. This would help explain the high rate at which bradykinin is metabolized. However, in other preparations, such as the rabbit aorta, it has been shown that a sustained contraction by bradykinin can also be achieved. A possible explanation for this difference is that degrading enzymes may not be present in high enough amounts to affect the action of bradykinin in these tissues.

In the guinea pig, ovokinin had an effect only on aortic rings precontracted with bradykinin, and with no other agent used in this study. Ovokinin potentiated the contraction by bradykinin ($p = 0.0008$) with a maximum effect of 108.7% ± 4.67, while the control ring immediately began declining toward baseline with 96.7% ± 1.78 of the maximum contraction at 2 minutes ($p = 0.0377$). In addition, when added to the bath of an

aortic ring which has not been previously contracted, ovokinin has no action of its own. These observations suggest that the effect of ovokinin in this particular preparation may involve mechanisms other than bradykinin receptor-mediated processes.

Angiotensin-converting enzyme (ACE), which is also known as kininase II, plays a major role in the degradation of bradykinin in many tissues. (Rouissi et al., 1990; Graf et al., 1992; Ersahin and Simmons, 1997). The use of captopril, an ACE inhibitor, was implemented in order to determine if ovokinin affects the metabolism of bradykinin. Incubation of guinea pig aortic rings with captopril for 30 minutes blocked the potentiation by ovokinin of the bradykinin contraction as well as increased the magnitude of the maximum contraction achieved. These data suggest that the mechanism of action of ovokinin involves a temporary reduction in bradykinin degradation. Ovokinin may competitively bind to the active sites of ACE, therefore allowing more available bradykinin to bind to receptor sites, activate second messenger pathways, and induce contraction (Figure 18). The effect of ovokinin is no longer observed after 3 minutes, which suggests that ovokinin may also be cleaved at a high rate.

If captopril had completely blocked bradykinin degradation in this assay, it might follow that the captopril-treated rings would be expected to result in a longer lasting contraction by bradykinin. However, the rings relaxed towards baseline in a timeline similar to the untreated rings. These results are consistent with data from another study by Hecker, Dambacher, and Busse(1992) on cultured human endothelial cells. They measured $[Ca]_i$ in cells with and without pretreatment by another ACE inhibitor, ramiprilat. The increase in intracellular Ca^{2+} levels stimulated by bradykinin was significantly increased after preincubation with ramiprilat. However, the duration of this increase remained the same as in untreated cells, with levels returning to baseline in approximately 180 seconds..

One possible explanation for this is that ACE was not completely inhibited. The effect of an ACE inhibitor varies across species and vessels, depending on the level of ACE activity and bradykinin efficacy in the tissue (Mombouli and Vanhoutte, 1992). In a study by Rhaleb et al. (1989), a concentration of 4.6×10^{-6} M captopril was used and was

sufficient to significantly potentiate the relaxation by bradykinin on the dog carotid artery. Rouissi et al. (1990) also used this concentration, incubated for 20-40 minutes, and indicated that this was the highest concentration that did not exert direct effects on isolated dog carotid artery, rabbit pulmonary artery, and rat portal vein. Even with a ramiprilat preincubation period of 90 minutes, as in the Hecker, Dambacher, and Busse (1992) experiment, there was no difference in the duration of the increase in $[Ca]_i$ as compared to untreated cells. In the present study, because captopril was added 30 minutes prior to stimulation by bradykinin at a concentration of 5×10^{-6} M, it seems unlikely that the transient contraction in captopril-treated rings is due to insufficient incubation time or concentration of inhibitor used unless the guinea pig aorta, in particular, has a significantly higher concentration of ACE than these other species, which is a possibility.

Another explanation for this transient contraction may be that other enzymes that also influence the degradation of bradykinin are present in the tissue. It has been shown that peptidases such as kininase I and neutral endopeptidase 24.11 (NEP) may be involved in bradykinin metabolism as well. However, they appear to play a much smaller role compared to ACE (Rouissi et al., 1990; Graf et al., 1992; Ersahin and Simmons, 1997). The presence of enzymes other than ACE in the guinea pig aorta is likely. However, one would suspect that if ACE was being completely inhibited by the captopril treatment, degradation by these other supporting enzymes would be much slower than the fairly rapid decline to baseline seen in the captopril-treated aortic rings which, again, was similar to that of the untreated vessels.

Desensitization of bradykinin receptors over time may also be a factor in the duration of contraction in the guinea pig aorta. Roscher et al. (1990) found that incubation of cultured human foreskin fibroblasts with bradykinin for 5-10 minutes resulted in decreased responsiveness to further bradykinin additions. This desensitization was associated with decreased prostacyclin release by bradykinin and a decrease in the maximum number of BK binding sites. They also used [^{3}H]-bradykinin in order to study the localization of the peptide as a function of time. An acid treatment succeeded in almost completely removing [^{3}H]-bradykinin bound to cell surface receptors and it was found that acid-resistant radiolabeled peptide could be measured increasingly with time until reaching

a steady state. An interpretation offered was that bradykinin-receptor complexes may be internalized in a time-dependent manner. This may act as a regulatory signal, perhaps affecting receptor down-regulation and desensitization.

In normotensive rat aortic rings, bradykinin had no effect. The inactivity of the peptide in this preparation was also reported in the review by Regoli and Barabe (1980). In addition, bradykinin has been shown to not exhibit any vasorelaxing activity in the thoracic aorta precontracted with noradrenaline in normotensive (Wistar) or spontaneously hypertensive rats (Wirth et al., 1996). However, this is in contrast to the results from a study by Berkenboom et al. (1995) where bradykinin was found to induce relaxation in Wistar rat aortas precontracted with phenylephrine (17.2 ± 3.1% relaxation of phenylephrine-induced contraction). Bradykinin also stimulated an increase in cytosolic free Ca^{2+} in cultured Wistar-Kyoto and SHR aortic smooth muscle which might suggest that B2 receptors are present on the smooth muscle of the aorta from these strains (Zhu et al., 1993). The B2 receptor has also been reported to be localized on the endothelial cells of the rat aorta using immunohistochemistry (Figueroa et al., 1995). This discrepancy in the the action of bradykinin in the rat aorta remains to be explained.

Interestingly, in aortic rings of spontaneously hypertensive rats, bradykinin induced a small but significant contraction, 0.39 g ± 0.047 (n = 10). Not much research has been conducted on the effects of bradykinin under resting conditions, or on aortas in particular since, in hypertension, the increase in peripheral vascular resistance is primarily regulated by arteries less than or equal to 200 μm in diameter while the large conduit arteries, like the aorta, play a limited role (Diederich et al., 1990). The majority of research has been centered on investigating altered endothelium-dependent relaxations by bradykinin in precontracted vessels from hypertensive animals. There is evidence that the relaxation by bradykinin is impaired even in young hypertensive rats. This suggests that the deficiency in bradykinin action is likely not a secondary effect of developing hypertension (Wirth et al., 1996). In addition to reduced vasorelaxation, the fact that bradykinin also stimulates a small level of contraction under resting conditions may also contribute to the development of hypertension in SHR.

In this study, there was also a significant difference in the level of contraction produced by the other contracting agents, phenylephrine, prostaglandin $F_{2\alpha}$, and potassium chloride in aortas of normotensive versus the spontaneously hypertensive rat. Larger contractions were achieved in normotensive aortic rings as compared to those from the spontaneously hypertensive rats. These results are in contrast to those of Wirth et al. (1996) in which they found that the magnitude of contraction by noradrenaline was larger in the hypertensive rats than age-matched normotensive rats. It seems logical that enhanced contraction by an α-agonist might contribute to increased peripheral resistance and blood pressure in the hypertensive animal. The fact that the normotensive rats (18 to 21 weeks) used in this present study were older than the SHR used (14 weeks) may possibly explain this discrepancy, as the contraction induced by noradrenaline has been shown to increase significantly with age in both normotensive (Wistar) rats and SHR (Wirth et al. 1996). In addition, there may exist possible genetic differences in the maximum contractions achieved, since Sprague-Dawley rats were used in this study as the normotensive control animal. One study by Wang, Sauve, and de Champlain (1995) measured the resting cytosolic free calcium concentrations in three different strains and found that levels in cultured rat aortic endothelial cells from Wistar-Kyoto and Sprague-Dawley rats were similar (96.3 ± 8.0 nM and 96.3 ± 10.7 nM, respectively) while that of SHR was significantly lower (50.4 ± 3.4nM). However, other differences may exist, so in order to make more accurate comparisons, age-matched Wistar rats should be used as the normotensive control to the SHR in future studies as was the case in the Wirth et al. (1996) experiments.

Ovokinin did not have a detectable effect on any of the normotensive rat preparations nor on spontaneously hypertensive rat aortic rings precontracted with bradykinin, phenylephrine, or potassium chloride. In addition, the effect of ovokinin on prostaglandin $F_{2\alpha}$-contracted SHR aorta at t = 2.5 minutes, the time point at which the maximum effect occurred, was not statistically significant. However, when comparing the ovokinin-treated and control curves (the percent maximum contraction as a function of time, see Figure 12), a statistically significant difference between the trends of these curves is seen ($p = 0.01$). The control ring slightly increased in contraction from time zero with a maximum effect at t = 2.5 minutes (104.7 ± 0.40%) while the ovokinin-treated ring

remained at approximately the same level (99.25 ± 1.95%) of contraction as was present at t = 0. These data may suggest that ovokinin has a very small vasorelaxing activity in this preparation. In future studies, it would be necessary to increase the sample size used in order to make a more confident conclusion about this particular preparation.

In conclusion, it appears that the effects of ovokinin are species- and tissue-dependent. The agent used to precontract the aortic ring also seemed to affect the action of ovokinin. This peptide generally did not have observable activity in the preparations tested in this study except a potentiation of the contraction by bradykinin in isolated guinea pig aortas. Although the effect of ovokinin treatment over time in SHR aortas precontracted with prostaglandin $F_{2\alpha}$ showed statistical significance, the difference in the level of contraction between ovokinin-treated and control rings at the maximum response time point was not significant. Further studies are needed to clarify whether ovokinin has any activity in this preparation. In the future, isolated tissue experiments involving other species and tissues, including the classical B1 rabbit aorta preparation, other arteries and veins as well as non-vascular smooth muscle preparations, are required in order to elucidate the actions and underlying mechanisms of ovokinin. Studies involving peripheral arteries in age-matched normotensive Wistar and spontaneously hypertensive rats might also be helpful in further understanding the physiological differences exhibited by the genetically hypertensive model.

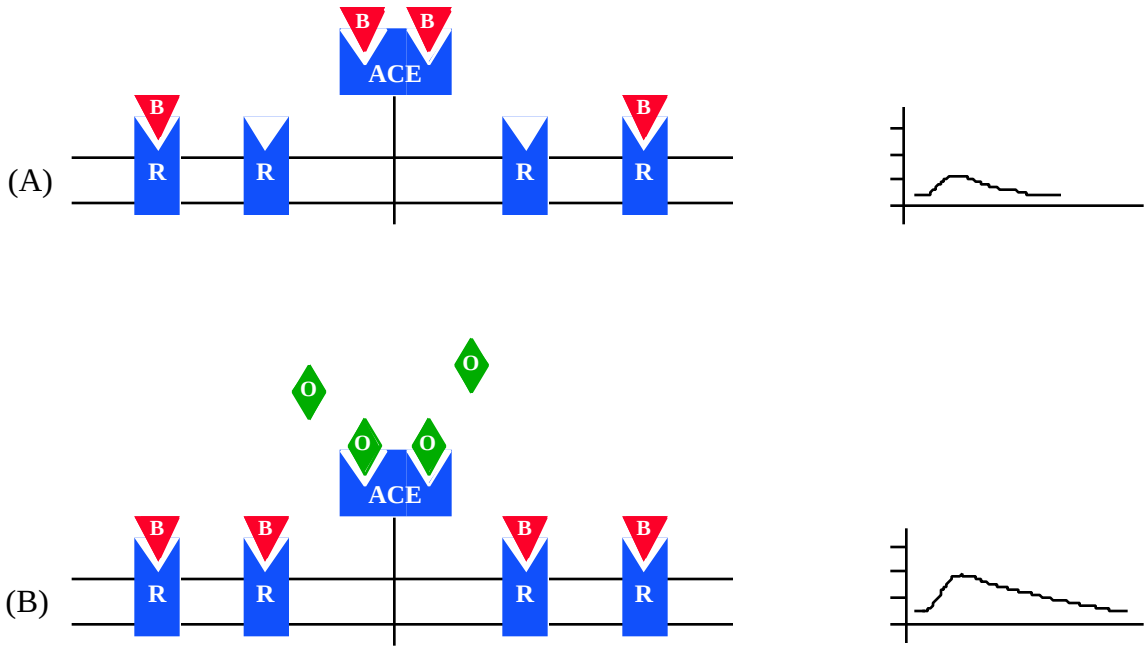

FIGURE 18: A schematic of a possible mechanism of action for ovokinin. (A): Bradykinin is binding to receptors, which results in contraction, but is also being degraded by the active sites of ACE. (B): Ovokinin is occupying ACE active sites thus allowing more bradykinin to bind to the receptors and inducing a greater contraction. B = bradykinin; R = bradykinin receptor; ACE = angiotensin converting enzyme; O = ovokinin.

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VITA

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Since August 1996, she has been enrolled in the Masters program at the Virginia-Maryland Regional College of Veterinary Medicine, focusing on vascular physiology under Dr. John C. Lee. Recently, she was awarded first place in the basic sciences category for her presentation at the Tenth Annual Research Symposium, VMRCVM.