THE EFFECTS OF XYLAZINE, DETOMIDINE, AND N^G-NITRO-L-ARGININE METHYL ESTER ON THE CANINE INTRAOCULAR PRESSURE

By

VIRGINIA A. SCHULTZ

Bachelor of Science
University of Central Oklahoma
Edmond, Oklahoma
1974

Doctor of Veterinary Medicine
Oklahoma State University
Stillwater, Oklahoma
1988

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
July, 1995

THE EFFECTS OF XYLAZINE, DETOMIDINE,

AND N^G-NITRO-L-ARGININE

METHYL ESTER ON THE

CANINE INTRAOCULAR

PRESSURE

Thesis Approved:

Thesis Advisor

Dean of Graduate College

ACKNOWLEDGEMENTS

I wish to thank Dr. Subbiah Sangiah for his encouragement and support throughout my veterinary and graduate education.

I wish to thank Dr. Art Quinn for his allowing the flexibility to combine my residency in ophthalmology with my graduate course work and to Dr. George Burrows for serving on my committee. I would like to thank Dr. Mark Payton, associate professor of statistics for his assistance with the statistical analysis of the data.

I extend thanks to Diana Moffeit for formatting my thesis.

Finally, to my husband, Randall Schultz, and our children, Josh and Amanda, thank you for the moral support and the understanding for all the time involved in my projects.

All of you have my sincere thanks.

TABLE OF CONTENTS

CHAPTER I

REVIEW OF LITERATURE

Intraocular Pressure

The canine eye is composed of three major parts; the globe, the optic nerve, and the accessory structures. The globe or eyeball's outer coat or tunic is fibrous to give the eye a rigid shape. It has a transparent anterior cornea and an opaque posterior sclera. The middle vascular tunic or uvea is composed of the pigmented iris, the ciliary body, and the choroid. The inner tunic is the light sensitive nerve layer termed the retina.

The anterior chamber is between the posterior surface of the cornea and the anterior surface of the iris. The posterior chamber lies between the posterior iris and the anterior lens surface (Figure 1).

The aqueous humor is the transparent fluid that fills the anterior and posterior chambers. The aqueous humor supplies nutrition to and removes waste products from the avascular cornea and lens. Aqueous is formed by the epithelium of the ciliary body. The mechanisms involved are diffusion, ultrafiltration, and active secretion (Gum 1991).

The vitreous humor fills the vitreous cavity which is the space posterior to the lens. The vitreous provides the support for the lens and also supports the retina against the choroid (Coulter, 1984).

The ciliary body is a highly vascular structure with a large surface area.

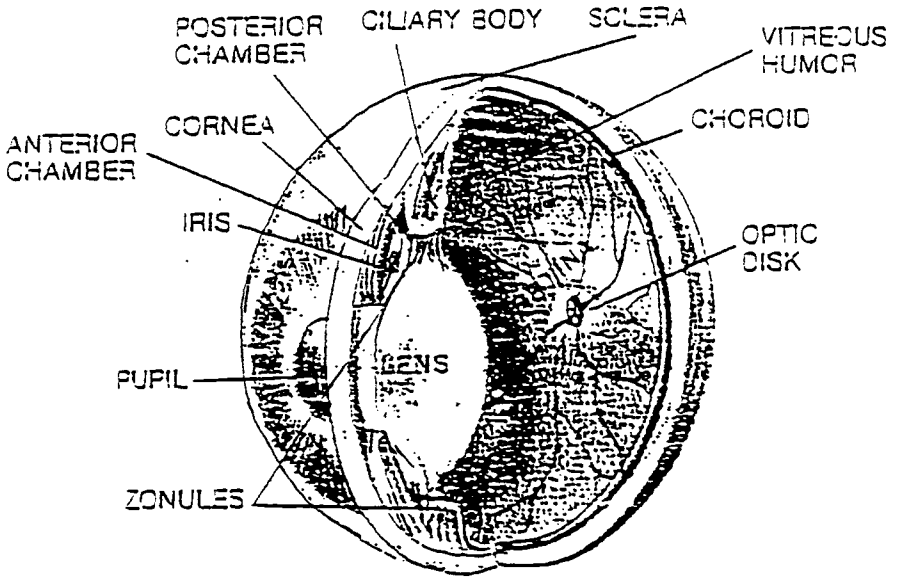

Figure 1. The Eye

(Renwick, 1994). As the aqueous exits the ciliary body, it enters the posterior chamber then the anterior chamber via the pupil. Due to the temperature difference between the cornea and the warm iris, aqueous humor circulates in an upward flow in the anterior chamber, ie convection.

The aqueous humor exits the globe via the trabecular meshwork at the iridocorneal angle, the area between the cornea and iris. It is then resorbed through the scleral venous plexus into the peripheral circulation. This is termed the conventional aqueous outflow pathway (Van Buskirk, 1979) and accounts for approximately eighty-five percent of the outflow.

The other fifteen percent of outflow has been described as an unconventional outflow pathway (Barrie, 1985). This unconventional or uveoscleral pathway involves diffusion through the iris, ciliary body, and sclerociliary cleft to eventually be resorbed by the choroidal and scleral circulation.

The amount of fluid within the globe is measured as the rigidity of the globe and is expressed as the intraocular pressure. The rate of aqueous formation should equal the rate of drainage. Intraocular pressure is measured by a variety of methods and expressed in millimeters of mercury,(mmHg). The intraocular pressure of the adult, healthy canine species is between 15-25 mmHg (Coulter, 1984). Normal intraocular pressure can also vary with age of the animal, restraint, positioning, breed, body condition, and many other factors (Renwick, 1993).

The aqueous outflow channels and trabecular meshwork are innervated by the sympathetic nervous system and provide a nervous influence on

intraocular pressure (Gwin, 1979).

An increase in intraocular pressure is due to an obstruction to outflow facility and not to hypersecretion of aqueous by the ciliary epithelium (Quinn, 1977) and an increase in intraocular pressure beyond the limits of health of the eye is the definition of glaucoma (Quinn, 1977).

Measurement of Intraocular Pressure

There are three methods of measuring intraocular pressure in the domestic animal. 1.Digital palpation is a crude estimation of intraocular pressure. 2.Indentation tonometry using a Schiotz tonometer (Schiotz®, Interstate) is a relatively easy procedure but the validity depends upon the patient, the clinician, and the instrument. 3.Applanation tonometry measures intraocular pressure by measuring the force required to flatten a constant area of the cornea (Gelatt, 1991b). The Mackay-Marg Model 12 applanation tonometer (Biotronics) provides an accurate estimation of intraocular pressure (IOP) in man and most domestic animals (Gelatt, 1991b).

With indentation and applanation tonometry, a topical anesthetic solution, proparacaine (Proparacaine®, Bausch and Lomb), is applied to the globe prior to tonometry. Proparacaine's main site of action is the nerve cell membrane where it interferes with the increase in membrane permeability to sodium ions and limits permeability through the lipid layer (Weisbecker, 1994). It does not influence IOP. Tonometry with the Mackay-Marg Model 12 applanation tonometer is then performed.

Drugs Used in Treatment of Glaucoma

Medical therapy for glaucoma involves either decreasing the inflow of

aqueous humor or increasing its outflow.

There are four primary classes of drugs used in the medical treatment of canine glaucoma (Lewis, 1989; Brightman, 1980; Gelatt, 1979). These include: (a) miotics, (b) adrenergic agents, (c) hyperosmotic diuretics, and (d) carbonic anhydrase inhibitors.

Miotics include parasympathomimetics such as pilocarpine, carbachol, demecarium, and echothiophate. These agents produce pupillary constriction, ciliary muscle contraction, and increase aqueous outflow through the trabecular meshwork (Gelatt, 1991a). Miotics also produce a vasodilation of blood vessels to enhance drainage of aqueous.

The adrenergic agents reduce IOP by increasing aqueous outflow by stimulating the alpha adrenergic receptors and blocking the beta adrenergic receptors to decrease aqueous production. Examples include epinephrine, depivalyl epinephrine (DPE), and timolol maleate. The decrease in IOP produced by adrenergic agents alone has not been sufficient to maintain glaucoma control. The side effect of contact blepharitits has also limited the use of epinephrine and DPE (Lewis, 1980).

The hyperosmotic diuretic agents such as mannitol and glycerin, are used for short term rapid reduction of IOP. These are primarily used prior to surgery to reduce intraocular volume.

Carbonic anyhdrase inhibitors limit carbonic anhydrase in the ciliary epithelium thus decreasing aqueous formation and lowering IOP (Lewis, 1980). The agents used in the canine patient include dichlorphenamide, acetazolamide, ethoxzolamide, and methazolamide. These are commonly used in combination

with the miotics.

Pharmacology of Alpha-2 Adrenergic Receptor Agonists

Xylazine hydrochloride (Rompun®, Haver) chemically is 2(2,6-dimethylphenylamino)-4H-5,6-dihydro-1,3-thiazine hydrochloride (Figure 2) with the code name Bay Va 1470 (Booth, 1982). Pharmacologically, it is classified as a sedative, a muscle relaxant, and an analgesic (Clark, 1969). However, it is not an anesthetic or tranquilizer. The Food and Drug Administration, FDA, has approved xylazine usage in the cat, dog, deer, elk, and horse in 2% and 10% solutions (Booth, 1982; Lumb, 1984).

Studies in cats and rabbits suggest that xylazine stimulates central alpha adrenoceptors that are distinct from peripheral alpha adrenoceptors (Loc, 1974). It is a potent antinociceptive agent (Schmitt, 1974) that is antagonized by the selective alpha-2 antagonist yohimbine (Yobine®, Lloyd Laboratories) (Hedler, 1981). Since yohimbine has been identified as a specific blocking agent at the presynaptic receptor region then xylazine's major target must also be the presynaptic alpha-2 region (Booth, 1982).

Muscle relaxation is mediated by the inhibition of intraneural transmission of central nervous system impulses (Lumb, 1984). Studies have shown that the effects of xylazine are not due to cholinergic, dopaminergic, histaminergic, or serotonergic pathways (Hsu, 1981; Schmitt, 1974).

Other documented effects of xylazine administration include: bradycardia (Haskins, 1986), a transient second-degree atrioventricular block (Kerr et al 1972), emesis-most commonly in the cat (Lumb, 1984), arterial hypotension due to the depressant effect on cardiac contractility and decreased cardiac

output (Gross et al, 1992; Antonaccio, 1973) and ventricular arrhythmias including fibrillations induced due to sensitization to epinephrine (Lumb, 1984).

A dose dependent hypothermia and an environmentally temperature dependent hyperthermia have been observed in cats (Lumb 1984). In rats and primates, when used in combination with ketamine (Ketaset®, Bristol), xylazine produces hypothermia.

Acute abdominal distension may occur in large dogs due to aerophagia or parasympatholytic activity (Booth, 1982). In the horse, when the head characteristically drops, there is decreased muscle tone with penile prolapse often occurring, as well as an increase in urination (England, 1992).

The ocular effects of xylazine have been described in the cat, rabbit, monkey, horse, and goat (Burke, 1986; Seleium, 1990; Kumar, 1976; Hsu, 1981). Effects ranged from prolonged palpebral and corneal reflexes (Kumar, 1976), to decreased intraocular pressure (Seleim, 1990; Burke, 1986; Trim, 1985; McClure 1976), unilateral miosis (Burke, 1986), and a dose-dependent mydriasis (Hsu, 1981). The ocular effects have not been described in the canine patient.

Detomidine hydrochloride (Dormosedan, Orion Corporation, Farmos) (Figure 3) chemically is 1 H imidazole, 4-[(2,3-dimethylphenyl)methyl]-hydrochloride. Pharmacologically, it is a non-narcotic sedative and analgesic with a dose dependent depth and duration (England, 1992). It has been approved by the FDA for use in mature and yearling horses in a 1% solution (England, 1992).

Clinically, with detomidine administration, heart rate is dramatically

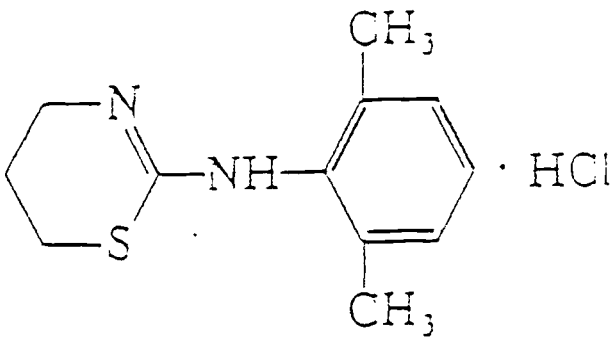

Figure 2. The Chemical Structure of Xylazine Hydrochloride

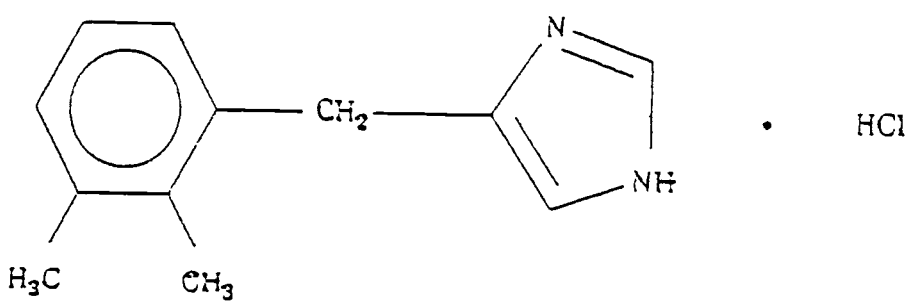

Figure 3. The Chemical Structure of Detomidine Hydrochloride

decreased with blood pressure elevated early then gradually returning to normal (England, 1992). Partial atrioventricular and sinoauricular blocks have been noted due to a change in cardiac muscle conductivity (Farmos).

Ocular effects have been studied in cats, rabbits, and isolated bovine ciliary process (Jin et at, 1991). Topically administered detomidine produced a dose dependent decrease in IOP in both cats and rabbits in both the treated and untreated eye (Jin et al, 1991). In the bovine ciliary process, the stimulated cyclic adenylate cyclase activity was decreased (Jin et al, 1991).

These studies suggest that detomidine also stimulates alpha-2 adrenoceptors because it is antagonized by selective alpha-2 adrenoceptor antagonists (England, 1992).

Xylazine hydrochloride and detomidine hydrochloride are both marketed as selective alpha-2 adrenergic receptor agonists. These agents exert their influence on the prejunctional alpha-2 adrenoceptors (Figure 4)(Burke, 1986) by a negative feedback system to regulate transmitter, ie norepinephrine, release during stimulation; thereby, attenuating response to lower levels of nerve stimulation (Burke, 1986). Norepinephrine is inhibited from stimulation of the alpha-1 receptors and subsequent production of aqueous humor (Hoffman, 1980; Starke, 1981; Berthelsen, 1977) (Figure 4).

Yohimbine (Figure 5) is an indole alkaloid found in nature. Chemically, it is a 17α-hydroxyyohimban-16α-carboxylic acid methyl ester (Goldberg, 1983; Gross et al, 1992; Hsu, 1983). It is a selective alpha-2 antagonist and prevents the autoinhibitory release of norepinephrine from the presynaptic nerve terminals by the alpha-2 agonists (Hsu, 1983).

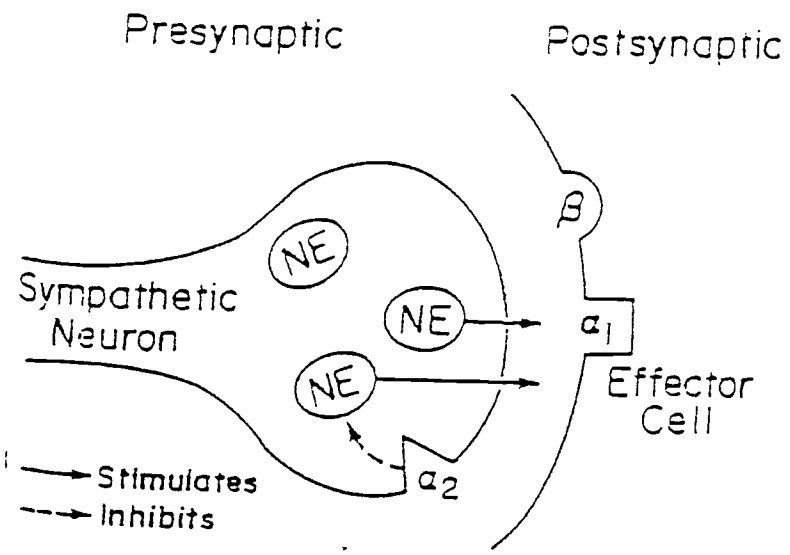

Figure 4. Alpha Adrenergic Receptors

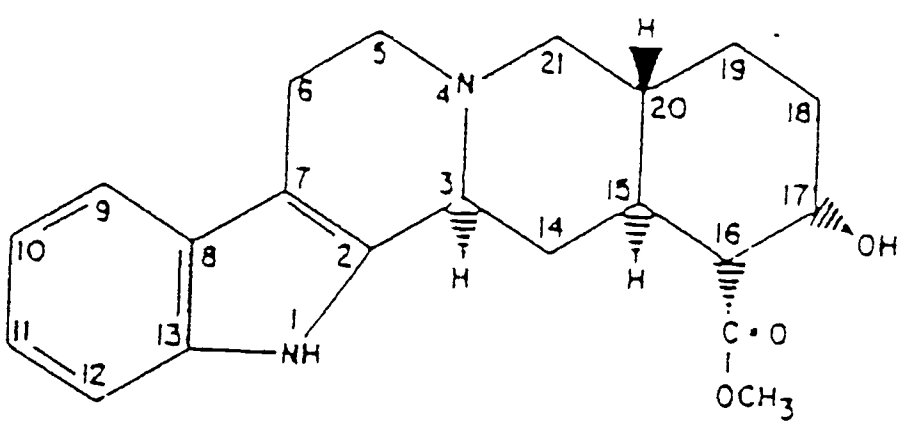

Figure 5. The Chemical Structure of Yohimbine

Currently, there are no studies available on simultaneous measurements of IOP and blood pressure with topically administered xylazine or detomidine in the dog to characterize the role of the alpha-2 adrenoceptor; therefore, it is the purpose of this study to provide this information.

Nitric Oxide-Regulation of Intraocular Pressure

Nitric oxide is a gaseous, tissue soluble intercellular, signaling molecule and an extracellular paracrine factor to carry information between cells (Nathanson, 1993; Sparrow, 1994). Nitric oxide (NO) is synthesized from the amino acid L-arginine by a group of enzymes, the nitric oxide synthases (NOS) via the L-arginine nitric oxide pathway (Moncada et al, 1989). NOS converts the guanidino nitrogen of L-arginine to L-citrulline, with the formation of equimolar amounts of NO (Marletta et al, 1988; Palmer et al, 1988; Bush et al, 1992).

Nitric oxide (NO) has recently been evaluated as a vasodilator and to play a role in IOP (Nathanson, 1993; Mandai et al, 1994). NOS exists as several isozymes, the two categories of NOS are constitutive (cNOS) and inducible (iNOS)(Fosterman et al, 1991). Cells that contain cNOS quickly and transiently produce small amounts of NO in response to agonists which raise intracellular Ca^{2+} concentrations, while cells with iNOS produce large amounts of NO for a prolonged period following a lag of several hours during which the enzyme is induced (Fosterman et al, 1991). The iNOS is the form that increases in uveitis (Mandai et al,1994) and which influences a decrease in IOP. iNOS is found in neutrophils, endothelial cells, vascular smooth muscle, and retinal pigment epithelium. The nitric oxide produced in endothelial cells relaxes blood vessels

by regulation of vascular tone and stimulates cytosolic guanylate cyclase (Nathanson, 1993)(Figure 6). By increasing blood flow and vascular permeability, the IOP is reduced with the increased aqueous outflow (Nathanson,1993; Mandai et al, 1994). Nitric oxide has a variety of other effects including mediation of macrophage cytotoxic effects (Sparrow, 1994). Nitric oxide has been studied in the retina of chick embryos (Zeevalk, 1994) and in Lewis rats with endotoxin-induced uveitis (Mandai et al, 1994) and may also promote the breakdown of the blood aqueous barrier (Mandai et al,1994). N^G-nitro-L-arginine methyl ester hydrochloride (NNA)(Figure 7) is a competitive NOS inhibitor and decreases nitric oxide production via NOS (Mandai,1994; Zeevalk, 1994).

Derivatives of L-arginine, such as N^G-methyl-arginine and N-nitro-L-arginine have been demonstrated to inhibit nitric oxide synthase in various biological tissues both in vivo and in vitro (Lechevalier et al, 1994; Mandai et al, 1994; Zeevalk, 1994). These compounds are commonly used as tools to investigate the role of endogenous nitric oxide in regulating various physiological and pathological conditions (Moncada et al, 1991; Moncada and Higg, 1993).

Nitric oxide synthase inhibitors have been shown to block the release of nitric oxide and increase mean arterial blood pressure in a dose dependent manner (Lechevalier et al, 1994) and depress the infiltration of polymorphonuclear cells in the anterior uvea in endotoxin-induced uveitis (Mandai et al, 1994).

Specific Objectives of the Proposed Research Project

There are at present no reports dealing with the effects of alpha-2 adrenergic receptor agonists such as xylazine and detomidine and NOS inhibitor on canine IOP and SABP. Therefore, the specfic objectives of this study are: 1: to characterize the effects of alpha-2 adrenergic agents such as xylazine and detomidine on IOP and SABP, 2: to characterize the effects of N^G-nitro-L-arginine methyl ester, an NOS inhibitor, on IOP and SABP, and 3: to characterize the interactions between xylazine and the N^G-nitro-L-arginine methyl ester on IOP and SABP in conscious dogs.

Specific Objectives of the Proposed Research Project

There are at present no reports dealing with the effects of alpha-2 adrenergic receptor agonists such as xylazine and detomidine and NOS inhibitor on canine IOP and SABP. Therefore, the specfic objectives of this study are: 1: to characterize the effects of alpha-2 adrenergic agents such as xylazine and detomidine on IOP and SABP, 2: to characterize the effects of N^G-nitro-L-arginine methyl ester, an NOS inhibitor, on IOP and SABP, and 3: to characterize the interactions between xylazine and the N^G-nitro-L-arginine methyl ester on IOP and SABP in conscious dogs.

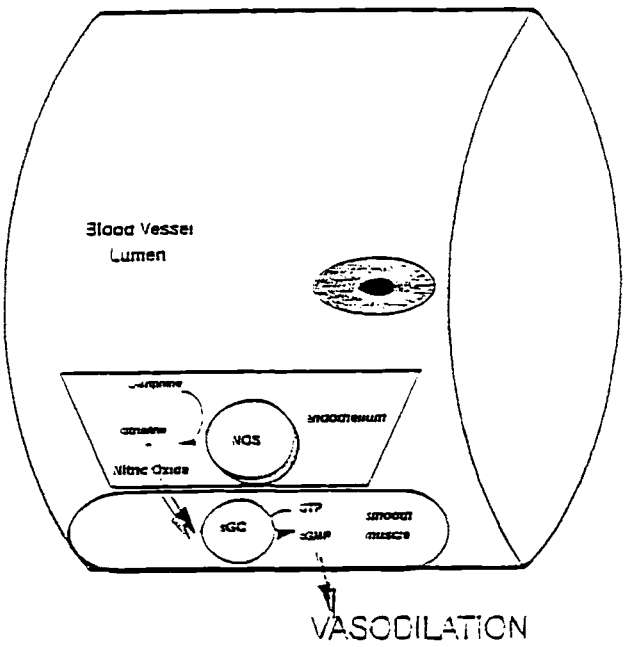

Figure 6. Biosynthesis of NO in the Endothelial Cell

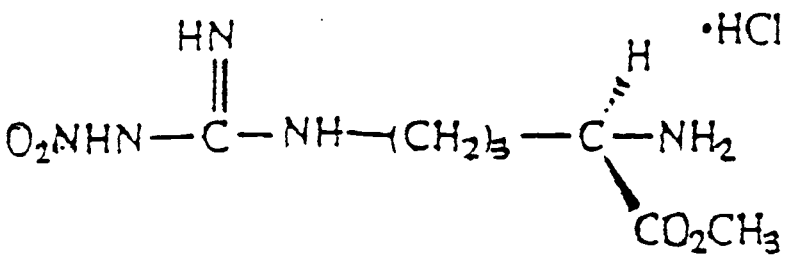

Figure 7. The Chemical Structure of N^G-nitro-L-arginine methyl ester

REFERENCES

Antonaccio, M.J., Robson, R.D. and Kerwin, L. (1973) Evidence for increased vagal tone and enhancement of baroreceptor reflex activity after xylazine (2-(2,6-dimethylphenylamino)-4-H-5,6-dihydro-1,3-thiazine) in anesthetized dogs. European Journal of Pharmacology 23, 311-315.

Barrie, K.P., Gum, G.G., Samuelson, D.A. and Gelatt, K.N. (1985) Quantification of uveoscleral outflow in normotensive and glaucomatous beagles by ^{3}H-labeled dextran. American Journal of Veterinary Research 46, 84.Berthelsen, S., and Pettinger, W.A. (1977) A functional basis for classification of alpha-adrenergic receptors. Life Sciences 21, 595-606.

Booth, N.H., and McDonald, L.E. (1982) Nonnarcotic analgesics. In: Veterinary Pharmacology and therapeutics. Iowa State University Press, Ames, 311-320.

Brightman, A.H. (1980) Pharmacologic management of glaucoma in the dog. Journal of American Veterinary Medical Association 177, 326-328.

Burke, J.A., and Potter, D.E. (1986) Ocular effects of xylazine in rabbits, cats, and monkeys. Journal of Ocular Pharmacology 2, 9-21.

Bush, P.A., Gonzalez, N.E., Griscalage,J.T., and Ignarro, L.B.(1992) Nitric oxide synthase from cerebellum catalyzes the fromation of equimolar quantities of nitric oxide and citrulline from L-arginine. Biochem. Biophys. Res. Commu. 185: 960-966.

Clark, K.W., and Hall, L.W. (1969) Xylazine. A new sedative for horses and cattle. Veterinary Record 85, 512-517.

England, G.C.W., Clarke, K.W., and Goosoens, L. (1992) A comparison of the sedative effects of three α_2-adrenoceptor agonists (romifidine, detomidine, and xylazine) in the horse. Journal of Veterinary Pharmacology and Therapeutics 15, 194-201.

Fornai, F., Blandizzi, C., and del Tacca, M. (1990) Central alpha-2 adrenoceptors regulate central and peripheral functions. Pharmacology Research 22, 541-554.

Fosterman, U., Schmidt, H.H.H.W., Pollock, J.S., Sheng, H., Mitchell, J.A., Warner, T.D., Kanane, M., Murad, F. (1991) Isoforms of nitric oxide synthase: characterization of purification from different cell types. Biochem. Pharmac. 1849-1857.

Garcia-Villar, R., Toutain, P.L., Alvinesie, M., and Ruckelbusch, Y. (1981) Pharmacokinetics of xylazine hydrochloride: an interspecific study. Journal of Veterinary Pharmacology and Therapeutics 4, 87-92.

Gelatt, K.N., Gum, G.G., Williams, L.W., and Barrie, K.P. (1979) Uveoscleral flow of aqueous humor in the normal dog. American Journal of Veterinary Research 40, 845-848.

Gelatt, K.N. (1991a) Canine glaucomas. In: Veterinary Ophthalmology Lea and Febiger, Philadelphia 396-428.

Gelatt, K.N. (1991b) Ophthalmic examination and diagnostic procedures. In: Veterinary Ophthalmology Lea and Febiger, Philadelphia. 195-237.

Goldberg, M.R., and Robertson, D: (1983) Yohimbine: a pharmacological probe for study of the α_2-adrenoreceptor. Pharmacological Reviews 35, 143-180.

Gross, M.E., Tranquilli, W.J., Thurmon, J.C., Benson, G.J., and Olson, W.A. (1992) Journal of American Veterinary Medical Association 201, 1887-1890.

Gwin, R.M., Gelatt, K.N., and Chiow, C.Y. (1979) Adrenergic and cholinergic innervation of the anterior segment of the normal and glaucomatous dog. Investigative Ophthalmology and Visual Science 18, 674-682.

Gum, G.G. (1991) Physiology of the Eye. In: Veterinary Ophthalmology. Lea and Febiger, Philadelphia. 124-161.

Haskins, S.C., Patz, J.D., and Farver, T.B. (1986) Xylazine and xylazine-ketamine in dogs. American Journal of Veterinary Research 47, 636-641.

Hedler, L., Stamm, G., Weitzell, R. (1981) Yohimbine. European Journal of Pharmacology 70, 43.

Hoffman, B.B., and Lefkowitz, R.J. (1990) Alpha-adrenergic receptor subtypes. New England Journal of Medicine 301, 1390-1396.

Hsu, W.H., Lee, P., and Betts, D.M. (1981) Xylazine-induced mydriasis in rats and its antagonism by alpha-adrenergic blocking agents. Journal of Veterinary Pharmacology and Therapeutics 4, 97-101.

Hsu, W.H., Betts, D.M., and Lee, P. (1981) Xylazine-induced mydriasis: possible involvement of a central postsynaptic regulation of parasympathetic tone. Journal of Veterinary Pharmacology and Therapeutics 4, 209-214.

Hsu, W.H. (1981) Xylazine-induced depression and its antagonism by alpha adrenergic blocking agents. Journal of Pharmacology and Experimental Therapeutics 218, 188-192.

Hsu, W.H. (1983) Effect of yohimbine on xylazine-induced central nervous system depression in dogs. Journal of American Veterinary Medical Association 182, 698-699.

Jin, Y., Verstappen, A., and Yorio, T. (1994) Characterization of α_2-adrenoceptor binding sites in rabbit ciliary body membranes. Investigative Ophthalmology and Visual Science 35, 2500-2508.

Jin, Y., Wilson, SII, Elko, E., and Yorio, T. (1991) Ocular Hypotensive effects of medetomidine and its analogs. Journal of Ocular Pharmacology 7, 285-96.

Kerr, D.D., Jones, E.W., Huggins, K., and Edwards, W.C. (1972) Sedative and other effects of xylazine given intravenously to horses. American Journal of Veterinary Research 33, 525-532.

Lewis, R.A., and Phelps, C.D. (1980) Medical therapy of glaucoma. Clinical Ophthalmology 3, 1-23.

Loc, T.Q., Tsoucaris-Kupfer, d., Bogaievsky, Y. Electroencephalographic studies in rabbits. Journal of Pharmacology 5, 51.

Lumb, W.V., and Jones, E.W. (1984). Preanesthetic Agents In: Veterinary Anesthesia Lea and Febiger, Philadelphia, 187-190.

Mandai, M., Yoshimura, N., Yoshida, M., Iwaki, M., and Honda, Y. (1994) The role of nitric oxide synthase in endotoxin-induced uveitis: effects of Ng-nitro L-arginine. Investigative Ophthalmology and Visual Science 35,3673-80.

Marletta, M.A., Yoon, P.S., Iyenger, R., Leaf, C.D. and Wishnok, J.S. (1988) Macrophages oxidation of FL-arginine to nitrite and nitrate. Nitric oxide is an intermediate, Biochemistry 29: 8706-8711.

McClure, R.J., Gelatt, K.N., Gum, G.G., and Manning, J.P. (1976) Effect of parenteral acepromazine and xylazine on intraocular pressure in the horse. Veterinary Medicine/Small Animal Clinician 1727-1730.

Moncada, S., Palmer, R.M.J., and Higgs, E.A. (1989) Biosynthesis of nitric oxide from L-arginine; a pathway for the regulation of cell function and communication. Biochem. Pharmac. 38: 1709-1715.

Moncada, S. and Higgs, E.A. (1993) The L-arginine-nitric oxide pathway. The New Eng. J. Med.329: 2002-2012.

Moncada, S., Palmer, R.M.J., Higgs, E.A. (1991) Nitric oxide: Physiology, pathophysiology and pharmacology. Pharmac. Rev. 43: 109-142.

Nathanson, J. (1993) Nitric oxide and nitrovasodilators in the eye: implications for ocular physiology and glaucoma. Journal of Glaucoma 2, 206-10.

Palmer, R.M.L., Ashton, D.S., and Moncada, S. (1988) Vascular endothelial cells synthesize nitric oxide from L-arginine. Nature 333: 664-666.

Quinn, A.J. (1977) Some observations on glaucoma. Oklahoma Veterinary Medical Association 29, 94-95.

Renwick, P.W. (1993) Glaucoma. In: Manual of Small Animal Ophthalmology British Small Animal Veterinary Association 193-212.

Schmitt, H., LeDouarec, J.C., and Petillot, N. (1974) Antagonism of the antinociceptive action of xylazine, an α-sympathomimetic agent, by adrenoceptor and cholinceptor blocking agents. Neuropharmacology 13, 295-303.

Seleim, M.A., Makady, F.M., and Ahmed, I.H. (1990) Intraocular pressure after xylazine and xylazine-ketamine injection in goats. Assiut Veterinary Medical Journal 24, 225-231.

Sparrow, J., Nathan, C., and Vodovotz, Y. (1994) Cytokine regulation of nitric oxide synthase in mouse retinal pigment epithelial cells in culture. Experimental Eye Research 59, 129-39.

Starke, K. (1981) Presynaptic receptors. Annual Review of Pharmacology and Toxicology. 21, 7-30.

Trim, C.M., Colbern, G.T., and Martin, C.L. (1985) Effect of xylazine and ketamine on intraocular pressure in horses. Veterinary Record 117, 442-443.

Van Buskirk, E.M. (1979) The canine eye: the vessels of aqueous drainage. Investigative Ophthalmology and Visual Science 18, 223-230.

Weisbecker, C.A. (1994) Alcaine. In: Physician's Desk Reference for Ophthalmology Medical Economics, Montvale, 207.

Yoshitomi, T., and Gregory, D.S. (1991) Ocular adrenergic nerves contribute to control of the circadian rhythm of aqueous flow in rabbits. Investigative Ophthalmology and Visual Science 32, 523-528.

Zeevalk, G. and Nicklas, W. (1994) Nitric oxide in retina: relation to excitatory amino acids and excitotoxicity. Experimental Eye Research 58, 343-50.

CHAPTER II

THE EFFECTS OF XYLAZINE, DETOMIDINE, AND N^G-NITRO-L-ARGININE METHYL ESTER ON THE CANINE INTRAOCULAR PRESSURE

Abstract

The effects of unilateral application of alpha-2 adrenergic receptor agonists such as xylazine and detomidine and N^G-nitro-L-arginine methyl ester to the left eye on intraocular pressure (IOP) and systemic arterial blood pressure (SABP) were determined in conscious dogs. Xylazine at 0.1, 0.3, and 1.0% concentrations produced a transient but significant reduction of IOP in both the eyes and SABP for a period of 30 minutes. Similarily unilateral application of 1.0% solution of detomidine produced a transient but significant reduction in IOP without altering the SABP. Pretreatment with 0.01% yohimbine solution produced a blockade of the 1.0% xylazine induced ocular hypotension. Unilateral application of N^G-L-arginine methyl ester (1.0, 5.0, and 10.0%) to the left eye has failed to alter both IOP and SABP in dogs. Results of this study provide additional evidence to support that the alpha-2 adrenoreceptors play an important role in the maintenance of IOP of the canine eye. Additional experiments are necessary to characterize the role of nitric oxide in IOP.

Introduction

Alpha-2 adrenergic agonists, such as xylazine and detomidine, have been used in veterinary medicine as sedatives, muscle relaxants, and analgesics

(Clark, 1969). Transient hypertension followed by prolonged hypotension, bradycardia, and heart block are some of the most important side effects of xylazine (Haskins, 1986; Gross et al, 1992). It has been demonstrated that topical administration of alpha-2 adrenergic agonists such as xylazine and detomidine produced a dose-dependent reduction in intraocular pressure in cats, rabbits, horses, and goats (Selium, 1990; Kumar, 1976; Hsu, 1985; McClure, 1976; Burke and Potter, 1986). Similarly, there is accumulating evidence to indicate that many of the alpha-2 adrenoreceptor agonists have been shown to produce a reduction in intraocular pressure in humans (Hodapp et al, 1981). It is interesting to note that apraclonidine (p-amino-clonidine) has been recently approved for therapy of acute glaucoma following laser surgery in humans.

Nitric oxide is a gaseous tissue soluble intercellular signaling molecule and an extracellar paracrine factor to carry information between cells (Nathanson, 1993; Sparrow, 1994). It has recently been evaluated as a vasodilator and to play a role in the regulation of IOP (Nathanson, 1993; Mandai et al, 1994). NO has been studied in the retina of the chick embryo and endotoxin induced uveitis in rats (Zeevalk, 1994; Mandai et al, 1994). Derivatives of L-arginine such as N^G-nitro-L-arginine methyl ester have been extensively used as tools in investigating the role of NO in various physiological and pathological conditions (Moncada et al, 1991; Moncada and Higgs, 1993). Studies concerning the role of alpha-2 adrenergic receptors and/or the interactions between alpha-2 adrenergic receptors and NO in the maintenance of IOP in dogs are very limited. The experiments outlined in this thesis are designed to investigate the effects of alpha-2 adrenergic agonists such as

xylazine, detomidine and N^G-nitro-L-arginine methyl ester, an inhibitor of nitric oxide, administered unilaterally to the left eye on intraocular pressure and systemic arterial blood pressure in dogs. In addition, the effects of alpha-2 adrenoreceptor blockade with yohimbine and NO sythase inhibition on xylazine induced reduction in intraocular pressure as well as systemic arterial blood pressure were determined.

METHODS AND MATERIALS

Drugs

Xylazine hydrochloride (Rompun®, Haver), detomidine hydrochloride (Dormosedan®, Orion Corporation, Farmos), proparacaine (Proparacaine®, Bausch and Lomb), yohimbine hydrochloride (Yobine®, Lloyd Laboratories), N^G-nitro-L-arginine methyl ester hydrochloride (Sigma Laboratories) were purchased commercially.

Animals

Five adult, healthy, male and female dogs weighing 12.0kg to 35.4kg were used in this study. Prior to use, the animals were examined for internal parasites and evidence of systemic and ocular disease. The animals were housed in indoor single unit kennels and maintained on commercial dog food. The animals were maintained on 12 hour light and 12 hour dark schedule with free access to food and water.

Drug Preparation and Dosage

All drugs were prepared in fresh, normal saline daily prior to the experiments. Xylazine hydrochloride was prepared as 0.1, 0.3, and 1.0% solutions. Detomidine hydrochloride was prepared as a 1.0% solution.

Yohimbine hydrochloride was prepared as a 0.01% solution. N^G-nitro-L-arginine methyl ester was prepared as 1.0, 5.0, and 10.0% solutions.

Experimental Protocol

Five dogs were randomly assigned to different drugs and different dosages within a given drug. Twenty-four hours were allowed to elapse between experiments with different doses of a single drug. Seven to ten days were allowed between experiments with different drugs.

Determination of Intraocular and Systemic Arterial Blood Pressure in Conscious Dogs

Each animal was individually taken to the examination room and one drop of proparacaine was applied to both eyes. The dog was placed on an examination table and a blood pressure cuff with monitor (Critikon-Dinamap tm-Veterinary Blood Pressure Monitor 8300®) was placed on the distal right forearm over the radial artery to monitor mean systemic arterial blood pressure (SABP).

The Mackay Marg Model 12 tonometer (Biotronics) was used to measure intraocular pressure (IOP). After anesthetizing the eyes with topical application of proparacanine, the IOP and the mean systemic arterial blood pressure (SABP) were monitored at 5 minute intervals for 15 minutes. After obtaining basal recordings of both IOP and SABP, one drop of the individual drugs at the chosen concentration was placed in the left eye and an equal volume of saline solution in the right eye. Both the IOP and SABP were monitored at 0, 5, 10, 15, 30, 45, and 60 minutes.

Experiments 1 to 3 were conducted to determine the effects of three different

doses (0.1, 0.3, and 1.0%) of xylazine hydrochloride on IOP and SABP.

Experiment 4 was conducted to determine the effects of a single dose (1.0%) of detomidine on IOP and SABP.

Experiment 5 was conducted to determine the effects of pretreatment (5 to 10 minutes) with yohimbine (0.01%) on xylazine (0.1%) effects on IOP and SABP.

Experiment 6 to 8 were conducted to determine the effects of 3 different doses (1.0, 5.0, and 10.0%) of N^G-nitro-L-arginine methyl ester on IOP and SABP.

Experiment 9 was conducted to determine effects of pretreatment with N^G-nitro-L-arginine methyl ester on xylazine (1.0%) effects on IOP and SABP.

Statistical Analysis

The intraocular pressure and systemic arterial blood pressure are presented as means +/- standard errors of the mean. These were subjected to ANOVA by SAS for repeated measures with each dose range and time interval. When differences were found to be significant, multiple comparisons with 0 time values and between doses were performed using LSD. $p < 0.05$ was considered significant.

RESULTS

I. Intraocular and Systemic Arterial Blood Pressure of Conscious Dogs

The intraocular pressures of both left and right eyes varied from 17.8 +/- 0.7 to 22.0 +/- 1.4 mm of Hg in unanesthetised, rested adult, healthy, conscious dogs of mixed sexes. The systemic arterial blood pressure as determined by the cuff method in conscious and rested dogs ranged from 76.0 +/- 1.9 to 115.5 +/- 9.0 mmHg.

II. Effects of Xylazine and Detomidine Hydrochloride on Intraocular Pressure and Systemic Arterial Blood Pressure

Topical administration of xylazine and detomidine to the left eye produced a significant reduction in intraocular pressure. Xylazine at 0.1%, 0.3%, and 10.0% produced a significant reduction in intraocular pressure beginning at 5 minutes, reaching a peak of 22.0% at 10 minutes and returning to the normal values of 0 time. The reduction in IOP doesn't appear to be dose dependent (Tables 1-3). However, the duration of the effect appears to be dose dependent with maximum effect lasting to 10 minutes at 0.1% and 30 minutes at 1.0% (Tables 1-3). Similarly,there is a significant reduction in intraocular pressure of the saline treated right eye following the application of xylazine at 0.1, 0.3, and 1.0% solutions to the left eye. Topical administration of xylazine at 0.1% to the left eye didn't significantly alter the systemic arterial blood pressure (Table 1). However, there was a transient reduction in SABP beginning at 5 minutes returning to control values at 60 minutes with 0.3% xylazine solution applied to the left eye (Table 2). Topical administration of 1.0% xylazine hydrochloride solution to the left eye produced a transient increase in SABP at 5 and 10 minutes followed by a significant decrease in SABP (Table 3). Topical application of detomidine hydrochloride solution at 1.0% produced a transient but significant reduction in IOP of the left eye from 21.5 +/- 1.5 mmHg at 0 time to 19.0 +/- 1.9, 17.0 +/- 1.3, and 18.0 +/- 2.0 mm of Hg at 0, 15, and 30 minutes respectively (Table 4). There was no significant change in the IOP of the saline treated right eye (Table 4). There

TABLE 1: Intraocular pressure (IOP) of the left (OS) and right (OD) eyes and systemic arterial blood pressure (SABP) following topical administration of xylazine (0.1%) solution to the left eye of five conscious dogs at various times.

Time (minutes)	IOP (mmHg) (Mean ± S.E.)		SABP(mmHg)
	OS	OD	Mean ± S.E.
0	17.8 ± 0.7[a]	18.8 ± 0.5[a]	88.4 ± 12.4
5	14.4 ± 0.5[b]	17.0 ± 0.4[a]	76.4 ± 2.0
10	13.4 ± 0.9[b]	16.0 ± 0.6[b]	81.0 ± 2.0
15	17.6 ± 1.2[a]	19.2 ± 1.0[a]	87.8 ± 4.5
30	18.4 ± 0.4[a]	18.8 ± 0.5[a]	92.2 ± 3.6

Means with the same superscripts are not significantly different.

TABLE 2: Intraocular pressure (IOP) of the left (OS) and (OD) right eyes and systemic arterial blood pressure (SABP) following topical administration of xylazine (0.3%) solution to the left eye of five conscious dogs at various times.

Time (minutes)	IOP (mmHg)	Mean ± S.E.	SABP(mmHg)
	OS	OD	Mean ± S.E.
0	19.4 ± 0.6[a]	19.4 ± 0.6[a]	99.4 ± 4.9[a]
5	15.6 ± 0.7[b]	16.6 ± 0.9[b]	78.0 ± 1.4[b]
10	14.8 ± 0.8[c]	15.8 ± 0.7[b]	83.0 ± 1.3[b]
15	15.0 ± 0.3[c]	15.6 ± 0.7[b]	90.2 ± 0.9[c]
30	14.8 ± 0.6[c]	16.2 ± 0.2[b]	89.4 ± 0.9[c]
45	15.8 ± 0.9[b]	17.2 ± 0.6[b]	89.0 ± 1.6[c]
60	19.2 ± 0.5[a]	19.2 ± 0.5[a]	98.5 ± 3.7[a]

Means with the same superscripts are not significantly different.

TABLE 3. Intraocular pressure (IOP) of left (OS) and right (OD) eyes and systemic arterial blood pressure (SABP) following topical administration of xylazine (1.0%) solution to the left eye of five conscious dogs at various times.

Time (minutes)	IOP (mmHg) (Mean ± S.E.)		SABP (mmHg)
	OS	OD	Mean ± S.E.
0	19.8 ± 0.8[a]	19.2 ± 0.7[a]	76.0 ± 1.9[a]
5	14.9 ± 0.4[b]	16.6 ± 0.6[b]	93.4 ± 3.3[b]
10	14.2 ± 0.5[b]	16.2 ± 0.6[b]	102.9 ± 3.4[b]
15	14.2 ± 0.6[b]	17.6 ± 1.0[b]	86.2 ± 2.1[c]
30	15.2 ± 0.5[b]	17.8 ± 0.9[b]	74.0 ± 5.4[a]
45	19.4 ± 1.2[a]	19.2 ± 0.5[a]	88.0 ± 1.5[c]
60	19.6 ± 0.7[a]	19.6 ± 0.7[a]	68.0 ± 6.0[a]

Means with the same superscripts are not significantly different.

TABLE 4: Intraocular pressure (IOP) of the left (OS) and right (OD) eyes and systemic arterial blood pressure (SABP) following topical administration of detomidine (1.0%) solution to the left eye of four dogs at various times.

Time (minutes)	IOP (mmHg) (Mean ± S.E.)		SABP (mmHg)
	OS	OD	Mean ± S.E.
0	21.5 ± 1.5[a]	20.8 ± 1.0[a]	115.5 ± 9.0
5	21.0 ± 3.3[a]	20.5 ± 2.5[a]	110.5 ± 9.1
10	19.0 ± 1.9[c]	17.5 ± 2.1[b]	93.7 ± 18.3
15	17.0 ± 1.3[b]	17.7 ± 1.9[b]	111.5 ± 7.1
30	18.0 ± 2.0[b]	18.0 ± 2.7[b]	93.5 ± 5.7
45	20.0 ± 2.9[c]	19.2 ± 3.3[a]	108.5 ± 7.3
60	19.5 ± 1.2[c]	20.0 ± 1.3[a]	113.5 ± 8.3

Means with the same superscripts are not significantly different.

was no significant change in the SABP when 1.0% detomidine hydrochloride was applied to the left eye for a period of 60 minutes monitored during the experiment (Table 4).

III. Effects of Alpha-2 Adrenoreceptor Blockade on IOP and SABP

Pretreatment of the left eye with 0.01% yohimbine solution for 5-10 minutes appeared to block the reduction in intraocular pressure induced by topical application of 0.1% xylazine solution to the left eye throughout the 30 minutes of recording period. However, there was no significant alteration in SABP (Table 5).

IV. Effects of Nitric Oxide Synthase Inhibitors on IOP and SABP

Topical administration of N^G-nitro-L-arginine to the left eye at 1.0, 5.0, and 10.0% solution failed to alter the IOP of both the eyes and systemic arterial blood pressures of conscious dogs for a period of 1 hour (Tables 6-8).

V. Interactions between the NO Synthase Inhibitor and Xylazine

Pretreatment with N^G-nitro-L-arginine methyl ester at 10.0% level to the left eye for 30 minutes failed to alter the reduction in IOP induced by 1.0% solution of xylazine hydrochloride at 5 to 30 minutes (Table 9).

TABLE 5: Intraocular pressure (IOP) of the left (OS) and right (OD) eyes and systemic arterial blood pressure (SABP) following topical administration of xylazine (0.1%) solution to the left eye of five conscious dogs pretreated (5-10 min) with 0.01% yohimbine solution at various times.

Time (minutes)	IOP (mmHg) Mean ± S.E.)		SABP (mmHg)
	OS	OD	Mean ± S.E.
0	18.4 ± 0.9	18.6 ± 0.6	78.6 ± 1.4[a]
5	18.2 ± 0.8	18.8 ± 0.5	82.8 ± 1.2[c]
10	17.6 ± 0.5	18.6 ± 0.6	65.0 ± 3.7[b]
15	16.8 ± 0.7	17.8 ± 0.5	97.6 ± 7.4[c]
20	16.2 ± 0.6	18.4 ± 0.6	92.0 ± 1.1[c]
25	17.8 ± 0.9	18.0 ± 0.6	88.6 ± 1.9[c]
30	18.6 ± 0.8	18.6 ± 0.6	98.6 ± 2.3[c]

Means with the same superscripts are not significantly different from each other.

TABLE 6. Intraocular pressure (IOP) of the left (OS) and right (OD) eyes and systemic arterial blood pressure (SABP) following topical administration of N^G-nitro-L-arginine methyl ester solution (1.0%) solution to the left eye of five conscious dogs at various times.

Time (minutes)	IOP (mmHg) (Mean ± S.E.)		SABP (mmHg)
	OS	OD	Mean ± S.E.
0	18.8 ± 1.3	18.4 ± 1.3	107.0 ± 5.3
5	19.2 ± 1.2	18.2 ± 1.3	107.0 ± 2.2
10	20.2 ± 1.2	19.2 ± 1.0	116.6 ± 5.9
15	21.2 ± 1.0	19.6 ± 1.5	108.4 ± 2.1
30	20.4 ± 0.9	19.6 ± 0.7	108.6 ± 5.4
45	20.0 ± 1.1	18.0 ± 1.3	113.6 ± 3.8
60	19.6 ± 0.7	18.4 ± 1.2	110.2 ± 2.9

TABLE 7. Intraocular pressure (IOP) of the left (OS) and right (OD) eyes and systemic arterial blood pressure (SABP) following topical administration of N^G-nitro-L-arginine methyl ester (5.0%) solution to the left eye of five conscious dogs at various times.

Time (minutes)	IOP (mmHg) (Mean ± S.E.)		SABP (mmHg)
	OS	OD	Mean ± S.E.
0	22.0 ± 1.4	22.0 ± 0.9	91.0 ± 7.7
5	22.0 ± 1.8	22.8 ± 1.2	104.8 ± 7.4
10	23.2 ± 1.3	21.6 ± 1.5	114.2 ± 10.6
15	22.4 ± 1.6	22.0 ± 1.1	100.4 ± 3.6
30	21.6 ± 1.2	21.2 ± 1.4	97.6 ± 6.2
45	23.2 ± 1.3	21.8 ± 1.1	94.2 ± 9.1
60	22.0 ± 1.5	21.2 ± 1.0	109.0 ± 9.7

TABLE 8. Intraocular pressure (IOP) of the left (OS) and right (OD) eyes and systemic arterial blood pressure (SABP) following topical administration of N^G-nitro-L-arginine methyl ester (10.0%) solution to the left eye of five conscious dogs at various times.

Time (minutes)	IOP (mmHg) (Mean ± S.E.)		SABP (mmHg)
	OS	OD	Mean ± S.E.
0	18.4 ± 1.2	17.2 ± 0.5	103.2 ± 7.3
5	18.4 ± 0.7	18.8 ± 1.5	113.6 ± 9.5
10	17.6 ± 0.7	17.6 ± 0.4	95.0 ± 10.6
15	17.6 ± 0.9	17.6 ± 0.7	108.6 ± 3.0
30	17.0 ± 1.3	17.2 ± 0.8	98.0 ± 10.4
45	18.4 ± 1.6	19.2 ± 0.8	103.0 ± 1.9
60	18.4 ± 1.2	18.8 ± 0.5	103.8 ± 4.9

TABLE 9. Intraocular pressure (IOP) of the left (OS) and right (OD) eyes and systemic arterial blood pressure (SABP) following topical administration of xylazine (1.0%) solution the left eye of five conscious dogs pretreated (30 minutes) with N^G-nitro-arginine methyl ester (10.0%) solution at various times.

Time (minutes)	IOP (mmHg) (Mean ± S.E.)		SABP (mmHg)
	OS	OD	Mean ± S.E.
0	22.0 ± 1.1[a]	20.4 ± 1.3[a]	117.4 ± 16.9
5	19.6 ± 0.9[b]	16.8 ± 1.6[b]	108.8 12.4
10	17.6 ± 0.9[c]	18.8 ± 1.6[b]	106.0 ± 5.6
15	17.6 ± 1.9[c]	17.2 ± 1.3[b]	101.8 ± 12.2
30	18.0 ± 1.8[b]	17.6 ± 0.7[b]	97.4 ± 7.3
45	21.2 ± 1.5[a]	20.8 ± 0.8[a]	108.6 ± 4.6
60	21.6 ± 0.9[a]	20.4 ± 1.2[a]	104.2 ± 3.7

Means with the same superscripts are not significantly different from each other.

DISCUSSION

The IOP of both left and right eyes and SABP of unanesthesized, rested adult, healthy, concious dogs of mixed sexes were 17.8 +/- 0.7 to 22.0 +/- 0.9 and 76.0 +/- 1.9 to 115.5 +/- 9.0 mmHg respectively. These values are consistent with the range of values previously reported (Edwards, 1985; Gelatt, 1991b).

Alpha-2 adrenergic agonists such as xylazine and detomidine appear to produce a dose dependent transient but significant reduction in IOP. Xylazine administered topically produced a significant reduction in IOP at 5 minutes reaching a peak of 22.0% at 10 minutes. The reduction in IOP doesn't appear to be dose dependent but the duration appears to be dose dependent with a maximum duration of 30 minutes with the 1.0% solution (Tables 1-3). There was a similar reduction of IOP of the saline treated right eye. These results are similar to those of other studies whereby unilateral topical xylazine administration lowered IOP bilaterally in unanesthetized rabbits, cats, and monkeys (Burke, 1986). Detomidine produced a significant but transient reduction in the IOP of the treated left eye from 21.5 +/-1.5 mmHg at 0 time to 19.0 +/- 1.9, 17.0 +/- 1.3 and 18.0 +/- 2.0 mmHg at 0, 15, 30 minutes respectively. There was no significant change in the IOP of the saline treated right eye. Our results are different from previous reported results in that detomidine has been reported to produce a significant bilateral decrease in IOP in rabbits and cats (Jin et al, 1991). This could be attributed to differences in

species.

The bilateral effects demonstrated by xylazine may be explained by two possible mechanisms: a CNS mediated effect and/or systemic transfer of the drug to the opposite eye that may be dose dependent (Burke, 1986). Other studies supporting the systemic transfer, dose dependent mechanism also have shown a unilateral miosis and a suppressive effect in the cat nictitating membrane of arterially administered xylazine (Burke, 1986).

Topical application of xylazine to the left eye produced a transient reduction in SABP at 5 minutes with 0.3% solution. With the 1.0% xylazine solution, there was a transient hypertension at 5 and 10 minutes followed by a significant hypotension. This is consistent with other reported studies of xylazine (Gross et al, 1992; Antonaccio, 1973). There are currently no reports on the pharmacokinetics of xylazine following its topical applicatioin to the eyes. It is possible that the systemic effects of xylazine on cardiovascular system following ocular application is mediated via some of the xylazine being carried from the site of application to the systemic circulation. It would be of great interest to characterize both the pharmacodynamic and pharmacokinetic profiles of drugs applied topically to the eyes. There was no significant change in SABP with topical 1.0% detomidine administration. This is in contrast to the decreased heart rate with hypertension then a gradual return to normal reported in mature and yearling horses (England, 1992). This may be explained by the possible route and amount of administraion -- topical versus intravenous or intramuscular. There is accumulating evidence to indicate the presence of a large number of alpha-2 adrenergic receptors in various ocular structures

particularly blood vessels (Mittag, 1985). The large number of alpha-2 adrenergic receptors present in the intraocular vascular bed have been demonstrated to regulate the vascular tone. A reduction in aqueous formation and/or increase in outflow have been observed when the vascular tone is reduced via activation of presynaptic alpha-2 adrenoreceptors with selective alpha-2 adrenergic agonists. Vasodilation of vessels in the ciliary processes could lower the ultrafiltration rate which could account for a decrease in aqueous humor formation (Innemee et al, 1981; Innemee and Van Zwieten, 1979; Langham, 1982; Lorenzetti, 1971).

Results of the present study provide additional support that the intraocular alpha-2 adrenergic receptors play an important role in modulating the intraocular pressure in the canine eye. It has been previously demonstrated that the reduction of intraocular pressure with adrenergic agents could possibly be mediated via an increase in cyclic AMP in the aqueous humor (Newfeld et al, 1972).

It has been further shown that the ocular hypotensive effect of medetomidine in rabbits and cats could be mediated via inhibition of adenylate cyclase in the ciliary processes (Jin et al, 1991).

Yohimbine is a selective alpha-2 adrenergic antagonist (Hsu, 1993). The bilateral decrease in IOP exhibited with topical unilateral administration of xylazine was eliminated when the treated eye was pretreated with yohimbine. Yohimbine antagonizes or blocks the alpha-2 agonist xylazine. This allows norepinephrine release which stimulates the post junctional alpha-1 receptors which in turn stimulates the ciliary body epithelium to continue aqueous humor

production and maintain a constant intraocular pressure.

A substantial body of basic research has established the importance of NO (EDRF-endothelium derived relaxing factor) in both basal and stimulated control of vascular tone. It has been demonstrated that the basal level of cyclic GMP are higher in vascular strips when the endothelium is intact than when it has been removed. It has been further shown that there is a basal release of NO from various blood vessels. A large number of studies have used L-arginine derivatives as inhibitors of NO synthase and demonstrated that the NO is the key mediator of vasodilation (Anderson et al, 1994). Nitrovasodilators such as nitroglycerine, amylnitrite, sodium nitroprusside, and hydralazine have been shown to produce either a increase or decrease in IOP in rabbits and monkeys respectively (Nathanson, 1993). Recent studies have demonstrated the existence of a NO-cGMP system in the segment of the eye (Nathanson, 1993). However, studies concerning the role of NO in the maintenance of IOP are at present nonexistent. Our results, although preliminary in scope, with the topical application of N^G-nitro-L-arginine methyl ester, an inhibitor of endogenous NO, failed to produce an alteration either in IOP or SABP. The failure of NO synthase inhibitor to produce any change in IOP and SABP could possibly be due to inability of the drug to reach the site of action or the thirty minute period following the application of the drug might not be sufficient time to inhibit the NO-cGMP system within the eye. Therefore, further studies are necessary before one could rule out the possible role of NO in the maintenance of IOP in the canine eye.

In conclusion, the results presented in this study provide evidence that

topical administration of alpha-2 adrenoreceptor agonists such as xylazine and detomidine produce a transient but significant reduction of IOP in the dog. Blockade of xylazine induced reduction in IOP with yohimbine indicates that alpha-2 adrenoreceptors in the eye play a role in the control of IOP. Further studies are necessary to characterize the role of NO in the maintenence of intraocular pressure.

CONCLUSIONS AND SUMMARY

Administration of α-2 adrenoreceptor agonists including xylazine and detomidine topically to the cornea caused a decrease in intraocular pressure in both eyes. The decrease in the contralateral eye's intraocular pressure was not as dramatic or as prolonged as the treated eye. With an increase in the concentration of xylazine, the IOP remained depressed for longer periods of time.

The decrease in intraocular pressure was eliminated when the selective alpha-2 antagonist yohimbine was administered prior to xylazine application.

The effect of both xylazine and detomidine on intraocular pressure can be explained by the fact that prejunctional alpha-2 agonists have autoinhibitory effects on norepinephrine release. This, in turn, causes a decrease in aqueous humor production and therefore lowered intraocular pressure. The use of yohimbine further explains these results. Yohimbine antagonizes or blocks the alpha-2 agonist xylazine. This allows norepinephrine release which stimulates the post junctional alpha-1 receptors which then stimulate the ciliary body epithelium to continue aqueous humor production and maintain a constant intraocular pressure.

The decrease in the pressure of the contralateral eye can be explained by the systemic transfer of drug that is also dose-dependent.

A large number of studies have used L-arginine derivatives such as N^G-L-

arginine as inhibitors of NO synthase and demonstrates that the NO is the key mediator of vasodilation. Topical application of N^G-nitro-L-arginine methyl ester to the left eye with an up to 10.0% solution failed to produce a significant alteration either in IOP or SABP in conscious dogs. Based on the results presented in this thesis, one could conclude that the α-$_2$ receptors are more susceptible to the effects of the alpha-2 agonists and additional experiments are necessary to support the role of NO in the maintenance of intraocular in the canine eye.

Although these were clinically normal dogs, it may be relevant to consider the use of an alpha-2 agonist in combination with the typical beta blockers or carbonic anhydrase inhibitors that are currently in use for glaucoma or increased intraocular pressure therapy.

REFERENCES

Anderson, T., Meredith, I., Ganz, P., Selwyn, A., and Yeung, A. (1994) Nitrovasodilators: similarities, differences, and potential interactions. Journal of American College of Cardiology 24, 555-66.

Antonaccio, M.J., Robson, R.D. and Kerwin, L. (1973) Evidence for increased vagal tone and enhancement of baroreceptor reflex activity after xylazine (2-(2,6-dimethylphenylamino)-4-H-5,6-dihydro-1,3-thiazine) in anesthetized dogs. European Journal of Pharmacology 23, 311-315.

Burke, J.A., and Potter, D.E. (1986) Ocular effects of xylazine in rabbits, cats, and monkeys. Journal of Ocular Pharmacology 2, 9-21.

Clark, K.W., and Hall, L.W. (1969) Xylazine. A new sedative for horses and cattle. Veterinary Record 85, 512-517.

Edwards, N.J. (1985) Cardiovascular system in Handbook of Veterinary Procedures and Emergency Treatment Philadelphia, W.B. Saunders.

England, G.C.W., Clarke, K.W., and Goosoens, L. (1992) A comparison of the sedative effects of three α_2-adrenoceptor agonists (romifidine, detomidine, and xylazine) in the horse. Journal of Veterinary Pharmacology and Therapeutics 15, 194-201.

Gelatt, K.N. (1991b) Ophthalmic examination and diagnostic procedures. In: Veterinary Ophthalmology Lea and Febiger, Philadelphia. 195-237.

Gross, M.E., Tranquilli, W.J., Thurmon, J.C., Benson, G.J., and Olson, W.A. (1992) Journal of American Veterinary Medical Association 201, 1887-1890.

Haskins, S.C., Patz, J.D., and Farver, T.B. (1986) Xylazine and xylazine-ketamine in dogs. American Journal of Veterinary Research 47, 636-641.

Hodapp, E., Kolker, A.E., Kass, M.A., Goldberg, I., Becker, B. and Gordon, M. (1981) The effect of topical clonidine on intraocular pressure. Arch. Ophthalmol., 99:1208.

Hsu, W.H. (1983) Effect of yohimbine on xylazine-induced central nervous

system depression in dogs. Journal of American Veterinary Medical Association 182, 698-699.

Innemee, H.C., deJonge, A.., van Meel, J.C. A., Timmermans, P.B.M.W.M. and van Zwieten, P.A. (1981) The effect of selective α_1- and α_2 adrenoreceptor stimulation on the intraocular pressure in the conscious rabbit. Naunyn-Schmiedebergs Arch. Pharmacol. 316, 294-8.

Innemee, H.C. and van Zwieten, P.A. (1979) The influence of clonidine on intraocular pressure. Doc. Ophthalmol. 46, 309-15.

Jin, Y., Verstappen, A., and Yorio, T. (1994) Characterization of α_2-adrenoceptor binding sites in rabbit ciliary body membranes. Investigative Ophthalmology and Visual Science 35, 2500-2508.

Jin, Y., Wilson, SII, Elko, E., and Yorio, T. (1991) Ocular Hypotensive effects of medetomidine and its analogs. Journal of Ocular Pharmacology 7, 285-96.

Kumar, A., Thurmon, J.C., and Hardenbrook, H.J. (1976) Clinical studies of ketamine HC1 and xylazine HC1 in domestic goats. Veterinary Medicine/Small Animal Clinician 1707-1713.

Langham, M.E. (1982) The intraocular pressure and pupillary response to the topical application of α-methyl norepinephrine and of α-methyl epinephrine. Invest. Ophthalmol Vis. Sci. 22 (ARVO Suppl.) 230.

Lorenzetti, O.J. (1971) Dose dependent influence of topically instilled adrenergic agents on intraocular pressure and outflow facility in the rabbit. Exp. Eye Res. 12, 80-7.

Mandai, M., Yoshimura, N., Yoshida, M., Iwaki, M., and Honda, Y. (1994) The role of nitric oxide synthase in endotoxin-induced uveitis: effects of Ng-nitro L-arginine. Investigative Ophthalmology and Visual Science 35,3673-80.

McClure, R.J., Gelatt, K.N., Gum, G.G., and Manning, J.P. (1976) Effect of parenteral acepromazine and xylazine on intraocular pressure in the horse. Veterinary Medicine/Small Animal Clinician 1727-1730.

Mittag, T.W. and Tormay, A. (1985) Adrenergic receptor subtypes in rabbit iris-ciliary body membranes; classification by radioligand studies. Experimental Eye Research 40, 239-249.

Mittag, T.W., Tormay, A., Severin, C., and Podos, S.M. (1985) Alpha-adrenergic antagonists: correlation of the effect on intraocular pressure and on α_2-adrenergic receptor binding specificity in the rabbit eye.

Experimental Eye Research 40, 591-599.

Moncada, S., Palmer, R.M.J., and Higgs, E.A. (1989) Biosynthesis of nitric oxide from L-arginine; a pathway for the regulation of all function and communication. Biochem. Pharmac. 38: 1709-1715.

Moncada, S., Higgs, E.A. (1993) The L-arginine-nitric oxide pathway. The New Eng. J. Med. 329: 2002-2012.

Nathanson, J. (1993) Nitric oxide and nitrovasodilators in the eye: implications for ocular physiology and glaucoma. Journal of Glaucoma 2, 206-10.

Seleim, M.A., Makady, F.M., and Ahmed, I.H. (1990) Intraocular pressure after xylazine and xylazine-ketamine injection in goats. Assiut Veterinary Medical Journal 24, 225-231.

Sparrow, J., Nathan, C., and Vodovotz, Y. (1994) Cytokine regulation of nitric oxide synthase in mouse retinal pigment epithelial cells in culture. Experimental Eye Research 59, 129-39.

Zeevalk, G. and Nicklas, W. (1994) Nitric oxide in retina: relation to excitatory amino acids and excitotoxicity. Experimental Eye Research 58, 343-50.

VITA

Virginia A. Schultz

Candidate for the Degree of

Master of Science

Thesis: THE EFFECTS OF XYLAZINE,DETOMIDINE, AND N^G-NITRO-L-ARGININE METHYL ESTER ON THE CANINE INTRAOCULAR PRESSURE

Major Field: Physiological Sciences

Biographical:

Personal Data: Born in Henryetta, Oklahoma, April 18, 1952, the daughter of Dwane and Betty Brittain. Married Randall Schultz March 16, 1973. Two children: Josh Randall Schultz, January 14, 1977 and Amanda Melissa Schultz, April 15, 1979.

Education: Graduated from Crooked Oak High School Independent School District in Oklahoma City, Oklahoma, May 1970; received Bachelor of Science degree in Education; major in Learning Disabilities; minor in Educationally Handicapped from the University of Central Oklahoma, July 1974; received the Doctor of Veterinary Medicine degree from Oklahoma State University, May, 1988; completed degree requirements for Master of Science degree at Oklahoma State University, July, 1995.

Professional Experience: Learning disabilities teacher, August 1974-1983; Teaching Associate, Veterinary Anatomy, Oklahoma State University,August 1988 to May 1994; Veterinary Practitioner in Small Animal Practice from May 1988 to present; Resident in Comparative Veterinary Ophthalmology at Boren Veterinary Medical Teaching Hospital, Oklahoma State University from August 1991 to December 1994.