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UNIVERSITY OF ALBERTA

DOPAMINE RECEPTOR ONTOGENY

BY

ROBERT JAMES PEARSON ©

A thesis submitted to the faculty of graduate studies and research in partial fulfillment of the requirement for the degree of

MASTER OF SCIENCE

IN

EXPERIMENTAL SURGERY

DEPARTMENT OF SURGERY

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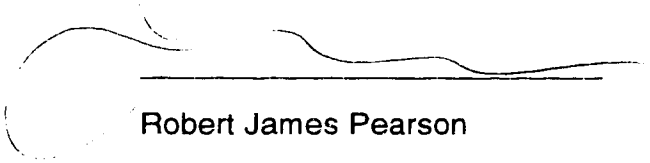

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ABSTRACT

Newborn responses to dopamine differ from the adult, possibly due to immature adrenergic receptors. Mesenteric and renal vascular responses to dopamine and fenoldopam (a selective DA_1 agonist) were assessed in the newborn piglet. Fenoldopam was used as a pharmacological probe for DA_1 receptors because dopamine is known to cross-react with alpha and beta receptors at higher dosages.

At initial surgery, two groups of 1 - 2 days old piglets (group 1 n = 16, group 2 n = 14) were instrumented with catheters in the external jugular vein and common carotid artery. Via a flank incision, ultrasonic perivascular flow probes were placed around the retroperitoneal superior mesenteric artery (SMA) and the left renal artery. Two days post-instrumentation measurements were recorded on conscious, non-sedated piglets in response to dopamine 2 - 32 µg/kg/min (group 1) or fenoldopam 1 - 100 µg/kg/min (group 2).

No significant renal vasodilatation was observed in response to dopamine or fenoldopam. The highest dosage (32 µg/kg/min) of dopamine produced a significant ($p < .05$) vasoconstriction, likely mediated by alpha receptors.

No significant mesenteric vasodilatation was observed with any dosages of dopamine (2 - 32 µg/kg/min) but with the highest dosage of dopamine (32 µg/kg/min) we observed a significant ($p < .05$) increase in blood flow, this was likely partly due to increases in blood pressure but a dopaminergic effect was also likely responsible in counteracting the known vasoconstrictory alpha effect. Fenoldopam produced significant ($p < .05$) vasodilatation of the mesenteric circulation at 5, 10, 25, 50 and 100 µg/kg/min.

Dopamine is <u>not</u> a renal or mesenteric vasodilator in the clinically used low dosage range. The functional immaturity of the DA_1 receptor response with an absence of a dopamine "protective effect" on these vascular beds suggests that other pressors should be considered and evaluated.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure **page**

LIST OF ABBREVIATIONS

BP - blood pressure

BUN - blood urea nitrogen

cAMP- cyclic adenosine monophosphate

Cl^- - chloride ion concentration

CNS - central nervous system

CO_2 - carbon dioxide

CV - cardiovascular

CVP - central venous pressure

DA_1 - peripheral dopamine receptor type 1

DA_2 - peripheral dopamine receptor type 2

D1 - central dopamine receptor type 1

D2 - central dopamine receptor type 2

DOP- dopamine

FENOLD - fenoldopam

GFR - glomerular filtration rate

GI - gastrointestinal

IMA - inferior mesenteric artery

IV - intravenous

I^{125} - radioactive iodine 125

K^+ - potassium ion concentration

MAP - mean arterial pressure

Na^+ - sodium ion concentration

PAH - para amino hippurate

PEEP - positive end expiratory pressure

PRU - peripheral resistance units

RENf - renal artery flow

RENr - renal artery resistance

%RENf - percentage change in renal artery flow from baseline

%RENr - percentage change in renal artery resistance from baseline

RVR - renal vascular resistance

SD - standard deviation

SMA - superior mesenteric artery

SMAf - superior mesenteric artery flow

SMAr - superior mesenteric artery resistance

%SMAf - percentage change in superior mesenteric artery flow from baseline

%SMAr - percentage change in superior mesenteric artery resistance from baseline

CHAPTER 1
INTRODUCTION

Dopamine is a naturally occurring endogenous substance which was first synthesized in 1910. It is now available as Intropin (Dupont-Pharma, Calgary, Alberta), Dopastat (Parke-Davis, Edmonton, Alberta) and Revimine (Rhone-Poulenc-Rorer, Montreal, Quebec) for the pharmacological treatment of shock and chronic refractory heart failure. This immediate precursor of norepinephrine and epinephrine possesses important intrinsic pharmacological properties. It is found in high concentrations in sympathetic nerves, adrenal medulla and in central and peripheral dopaminergic nerves. Dopamine is a substrate for both monoamine oxidase and catechol-o-methyltransferase and thus is ineffective when administered orally because of first pass liver metabolism. Clinically it is used most often as an intravenous infusion because of its short half life of 2 - 3 minutes(1).

In 1974, Goldberg(2) published a landmark review article in the New England Journal of Medicine which emphasized the uniqueness of this catecholamine. Dopamine has hemodynamic properties which are not duplicated by the other sympathomimetic amines. It has a unique ability to cause both relaxation and contraction of vascular smooth muscle (Appendix 1). The predominant effect depends on the vascular bed studied and the dosage administered. It has been shown in adult humans that dopamine in low dosages increases the renal blood flow, urine output and sodium excretion without affecting systemic blood pressure(3, 4). This effect on renal blood flow has been most useful clinically.

Following the initial demonstration of specific renal vasodilatation by dopamine, Eble(5) reported that dopamine caused similar vasodilatation in the mesenteric vascular bed. Scheulke et al(6) and Von Essen(7) later demonstrated vasodilatation in the coronary and cerebral vascular beds respectively (Appendix 2).

The dose response properties of dopamine give it an advantage over the more conventional pressors (i.e.. Norepinephrine, epinephrine and isoproterenol). In comparison with the drugs that act on Beta adrenergic receptors in the heart and blood vessels, dopamine did not dilate the skeletal muscle vascular beds, did not cause a great increase in heart rate and did not cause a decrease of blood flow to the kidney(2, 3).

The popularity of dopamine as a pharmacological pressor arises from its potent effects on the cardiovascular system with a paucity of adverse effects, the most significant being arrhythmias. The first line pressor in most intensive care settings is dopamine.

In the past 20 years there has been significant advancements made in the support of the critically ill newborn. It is likely that exogenous catecholamine support has played a significant role in this progress. Although dopamine is well accepted as a first line pressor in adults its role in the newborn is not well documented or understood.

Children are not physiologically small adults and there is growing evidence that the cardiovascular system of the immature animal responds differently than the adult. In 1979, Driscoll(8) reported his reluctance to use dopamine first line as a pressor in children less than 6 months of age because of laboratory animal data that suggested that dopamine may be relatively ineffective if ventricular myocardial sympathetic innervation is incomplete and that dopamine may detrimentally increase pulmonary

vascular resistance. Indeed others have questioned the clinical use of dopamine in the newborn.

Although little is known about the developmental pharmacology of dopamine in infants (Appendix 3), this thesis will examine the background literature available which addresses the mesenteric, renal, and systemic vascular effects of dopamine in a newborn model. In addition, the measured effects of dopamine and fenoldopam (specific DA_1 agonist) on the renal and mesenteric vasculatures of the newborn piglet are reported and the results discussed.

Chapter 2

PERIPHERAL DOPAMINE RECEPTORS

To understand how adrenergic agents function the known catecholamine receptors must be reviewed. These receptors are usually cell membrane associated glycoproteins with high affinity for the specific catecholamine.

Historically, in 1948 Ahlquist(9) recognized two distinct populations of adrenergic receptors, which he termed Alpha and Beta based on the physiological response obtained after administration of different catecholamines. Since then these receptors have been subdivided. Lands et al(10) described $Beta_1$ and $Beta_2$ adrenergic receptors and then the Alpha-adrenergic receptors were divided into $Alpha_1$ and $Alpha_2$ by Berthelson et al(11).

Before the discovery of vascular dopaminergic receptors more than 15 years ago, vasodilatation was thought to be largely confined to the $Beta_2$ adrenergic receptor mediated response. The peripheral vascular dopaminergic receptors have been subdivided into DA_1 and DA_2 by Goldberg(12). This separate classification of dopaminergic receptors was needed to distinguish them from the central dopaminergic receptors of Kebabian and Calne(13).

The dopamine receptors in the CNS and certain endocrine organs are classified into D_1/D_2 subtypes. Because of the importance of dopaminergic mechanisms in, for example, Parkinson's disease and

schizophrenia, the study of central receptors is much more advanced than peripheral receptors. The central dopamine receptors have been characterized by electrophysiological, behavioral, and biochemical techniques. The D_1 receptors are linked to adenylate cyclase and the D_2 receptors are not linked to adenylate cyclase(13). The D_1 receptors are located post synaptically and the D_2 receptors can be post or presynaptic in location. In addition the D_2 receptor has been solubilized, purified(14) and cloned(15). There has also been some progress in purifying the D_1 receptor(16).

There are similarities and differences between the D_1/D_2 and the DA_1 and the DA_2 receptors. It seems that the central receptors have a higher affinity for dopamine than the peripheral receptors(16, 17). Like the central receptors the DA_1 receptors are usually postsynaptic in location and the DA_2 receptors may be located at both pre and postsynaptic sites(18, 19).

The second messenger system activation appears to be the same for D_1 and DA_1 receptors, that is the occupation of D_1 and DA_1 receptors leads to stimulation of adenylate cyclase activity(20, 21). With the DA_2 receptor, as with the D_2 receptor, there may(19) or may not(22) be an associated inhibition of adenylate cylase activity. The DA_1 and DA_2 receptors have yet to be purified, sequenced and cloned.

The most significant development in the characterization of the peripheral dopamine receptors has been the chemical synthesis of specific selective DA_1 and DA_2 agonists and antagonists which will be discussed in the next chapter. These breakthroughs allowed localization and characterization of these receptors via radioligand binding and pharmacological studies. The prototype DA_1 receptor is found in the renal

artery and is associated with vasodilatation(12, 23). The DA_2 receptor is associated with inhibition of presynaptic norepinephrine release(12, 23).

In the vasculature of the kidney and bowel there are probably only the two above mentioned dopamine receptor subtypes. However, dopamine has a differential selectivity for Alpha and Beta adrenergic receptors which are also present in the vascular smooth muscle. This complicates the pharmacological picture.

In the past it was difficult for the pioneers in this area to conceptualize a specific dopamine receptor as being responsible for the vasodilatory phenomenon that was observed in selected vascular beds. For example, in the renal vascular bed Goldberg(3) observed in adult dogs that there was a biphasic response (vasodilator/vasoconstrictor) action of dopamine, this presented him with the difficult challenge in characterizing a single receptor that explained this observation. Fortunately, the vasoconstrictor component appeared to be mediated by Alpha adrenergic receptors and could be eliminated by sufficient dosages of phenoxybenzamine(24). After alpha receptor blockade, there was a dopamine dosage related increment in renal blood flow. The same experiment was carried out in the femoral vasculature, but here after alpha blockade no increase in blood flow was demonstrated by dopamine infusion. This led Goldberg to believe that dopamine was acting on more than one type of receptor.

The mechanism of vasodilatation caused by dopamine is clearly a postsynaptic phenomenon as the promptness of the effect and the lack of tachyphylaxis suggest that it is a direct effect on receptors and not secondary to release of other endogenous materials. In addition, repeated studies after administration of reserpine and hexamethonium have demonstrated that vasodilatation is not affected by these procedures(24). The vasodilatation is

not secondary to release of prostaglandins since it is not affected by pretreatment with indomethacin(25); in fact, vasodilatation is increased as a result of pretreatment with indomethacin(26).

The differential selectivity of dopamine for different receptor types allows an understanding of the pharmacological properties of the drug. Table 3 demonstrates a variable dosage dependent effect of dopamine on the various know receptors. Clearly the vasoconstrictor "Alpha effect" predominates with high dosage dopamine.

The effects of dopamine on Beta-adrenergic receptors in vascular tissue is controversial. Studies in the dog(3) suggest that dopamine possesses weak Beta$_2$-adrenergic activity. On the other hand, no Beta-adrenergic relaxation was observed in isolated mesenteric, renal, or coronary arterial strips(27, 28). Since the latter strips are extremely sensitive to Beta-adrenergic activation, the data suggests that if there is a dopamine effect on Beta-adrenergic receptors, it must be very weak. Despite being an agonist at Alpha and Beta-adrenergic receptors, dopamine is 10 to 100 times less potent than norepinephrine at these sites. Furthermore, dopamine may condense with aldehydes to form various biologically active compounds. For example, tetrahydropapaverolines are potent Beta-adrenergic receptor agonists and tetrahydroisoquinolines may have Alpha-adrenergic receptor stimulating properties(29). It is important to consider these pharmacological properties.

CHAPTER 3

SYNTHESIS OF PERIPHERAL DOPAMINE RECEPTOR AGONISTS AND ANTAGONISTS

The existence of phentolamine as a selective Alpha receptor antagonist has allowed the elimination of dopamine's Alpha effect in experimental models. Propranolol is also available as a $Beta_1$ and $Beta_2$ receptor antagonist. Pretreatment with both the Alpha and Beta antagonists should allow elimination of the Alpha and Beta effects of dopamine.

The most recent significant development in dopamine receptor pharmacology has been the synthesis of specific and selective DA_1 and DA_2 agonists and antagonists. These are "specific" in the sense that the compounds, within a certain dosage range, act only on DA receptors without action on Alpha, Beta, serotonin, acetylcholine, or other known receptors. "Selective" is the term used to differentiate the relative activity of a compound on DA_1 or DA_2 receptors.

DA_1 Agonist:

In the early 1980's the Smith Kline and French laboratories in the United States synthesized an extraordinarily selective, specific, and potent DA_1 agonist called Fenoldopam (SK & F 82526).

This benzazepine derivative has been used as a DA_1 agonist in several experimental studies(30, 31, 32, 33, 34, 35). Animal studies have shown that single oral or intravenous dosages of fenoldopam produce dosage-dependent increases in renal blood flow and decreases in total peripheral resistance and mean arterial blood pressure. Fenoldopam, like

dopamine does not cross the blood brain barrier(33). Fenoldopam is a more potent stimulator of dopaminergic receptors than dopamine itself(36). Fenoldopam has been used in human patients with mild to moderate essential hypertension, at a dosage of 100 mg. orally, this led to significant reductions in both systolic and diastolic blood pressures, associated with increases in renal blood flow, urine volume, and sodium excretion(37). In this study, patients were maintained on 100 mg. four times a day for at least 3 weeks. During this treatment interval, there appeared to be a sustained effect on both systolic and diastolic blood pressure. The acute hemodynamic decrease in blood pressure seen with fenoldopam appeared attenuated after 4 - 6 weeks of therapy, this tachyphylaxis is not understood and requires further investigation. It is hoped that fenoldopam may have a clinical role as a renal and systemic vasodilator and is proposed for the treatment of hypertensive emergencies by IV route(36).

Minimal systemic hemodynamic effects of fenoldopam are observed at dosages less than 0.2 μg/kg/min (36). Poinsot (38) demonstrated in adult human patients requiring mechanical ventilation with PEEP that renal hemodynamics could be improved with fenoldopam at a dosage of 0.2 μg/kg/min probably mediated by an increased kidney perfusion secondary to renal artery vasodilatation.

In newborn animal models the dosages of fenoldopam used have been much higher suggesting a reduced sensitivity of the DA_1 vasodilatory mechanism in the newborn. Fenoldopam has been reported to be selective for the DA_1 receptors at dosages from 0.1 to 100 μg/kg/min(30,31). One study in newborn lambs used fenoldopam in a dosage of 60 μg/kg/min(32) to decrease mean systemic arterial pressure.

Kohli(39) reported, in adult dogs, that there was some cross-reactivity of fenoldopam with alpha receptors at higher dosages. Alpha receptor blocking activity was not observed with the 0.1, 0.2, and 2 μg/kg/min infusions of fenoldopam but the 5 μg/kg/min infusion produced a 14% inhibition of the vasoconstrictor effect of phenylephrine. He concluded that fenoldopam decreases renal vascular resistance in dosages which are 25 to 50 times less than the dosage needed to antagonize alpha receptors.

DA_1 Antagonist:

In the past all DA_1 antagonists studied blocked DA_2 receptors either at much lower dosages or at a dosage close to that required to block DA_1 receptors. A breakthrough in this area came in 1983 with the synthesis of SCH23390 a selective DA_1/D_1 receptor antagonist reported by Iorio et al(40). This drug provided a revolution in the identification and the physiological and behavioral functions of DA receptors. Despite its experimental use, SCH23390 was unsuitable for clinical development because it has an unacceptably short duration of action in the primate. A new second generation benzazepine, SCH39166 was synthesized in 1988 by the Schering-Plough Corporation, and possesses the same pharmacological profile of SCH23390 but has a duration of action of at least 6 hours(41).

DA_2 Agonists and Antagonists:

In contrast to the rigid chemical requirements for DA_1 agonists and the relatively few compounds which are active at that receptor, a great number of compounds with markedly diverse chemical structures have been shown to be active on DA_2 receptors. Indeed, it is reported that it is difficult

to determine a common requirement for activation of DA_2 receptors. Compounds with chemical structures as diverse as dopamine, piribedil, and LY14876 act as DA_2 agonists(12).

Most dopamine antagonists block DA_2 receptors at lower dosages than DA_1 receptors, and thus, it is relatively easy to selectively block DA_2 receptors. Domperidone is usually used because when administered in a dosage 1000 times the dosage required to block DA_2 receptors, it has no effect on DA_1 receptors(42).

The commercial availability of these selective D_1/DA_1 and D_2/DA_2 agonists and antagonists has allowed identification of different dopamine receptor subtypes in many tissues. Using radioligand binding studies with radiotracers attached to specific DA_1 antagonists, these receptors have been described in renal, splanchnic, coronary and cerebral vascular smooth muscle as well as the adrenal cortex(43). DA_2 receptors are found in the carotid body, gastrointestinal tract, and anterior pituitary gland. With the identification of DA receptors in the CNS it has been suggested but not yet widely accepted that receptors should not be classified according to location and Goldberg has discontinued the practice of comparing peripheral and central dopaminergic receptors(12). Studies with selective agonists and antagonists have tended to bring together the DA_1, DA_2 and D_1, D_2 classifications. The measurement of adenylate cyclase activity and ligand binding assays using these selective antagonists and agonists allow a more meaningful comparison between the central and peripheral receptors. Nevertheless, several discrepancies and more research is needed before the receptors (central and peripheral) are considered identical. The discrepancies may eventually be found to be due to differences in methodology rather than true receptor differences, that is, previous

comparisons were hampered by the use of non-selective agonists and antagonists. Before these ligands can be used as receptor probes their specificity for a specific organ needs to be established because the affinities of these ligands may differ from organ to organ. It seems that in the kidney as well as the mesenteric vasculature that there are probably only two dopamine receptor subtypes and the specificity of these ligands has been previously established(12).

CHAPTER 4

DOPAMINERGIC EFFECTS IN THE RENAL AND MESENTERIC VASCULATURES

The renal circulation

In the kidney, the vasodilatation induced by dopamine is mainly due to occupation of postsynaptic DA_1 receptors, since the effect persists during Alpha adrenergic blockade and is mimicked by DA_1 agonists and blocked by DA_1 antagonists(44). The vasorelaxing properties of dopamine and DA_1 agonists have been demonstrated in the main renal, arcuate, and interlobular arteries, and afferent and efferent arterioles. In vitro, DA_1 agonists induce vasorelaxation of the afferent and efferent arterioles to a similar degree. Presynaptic DA_2 receptors, which inhibit the neuronal release of norepinephrine, can also induce renal vasodilatation in a rodent model(18) but Goldberg, working with a dog model, has never found an increase in renal or mesenteric blood flow using DA_2 agonists(12). The complete function of the postsynaptic renal vascular DA_2 receptor is not yet established.

The major receptor subtype mediating renal vasodilatation is the DA_1 subtype, whereas the DA_2 subtype predominates in glomeruli and may directly modulate glomerular filtration rate. Both receptors are present in the brush border and basolateral membranes of the proximal convoluted tubule and cortical collecting duct.

In addition to increasing renal blood flow up to 50% in adults, exogenous dopamine generally increases urine flow, sodium, chloride, potassium, phosphate, and calcium excretion. In fact, renal sodium excretion may increase by up to 500%(2). The bulk of the evidence indicates that occupation of the DA_1 receptor directly decreases renal tubular sodium transport; whereas occupation of DA_2 receptors may increase sodium transport. Dopamine may increase water excretion by inhibiting the release of antidiuretic hormone from the posterior pituitary gland as well as suppression of aldosterone secretion by the adrenal cortex via stimulation of the adrenal DA_1 receptors(43, 44).

Endogenous dopamine (via DA_1 receptors) probably plays a role in regulation of renal sodium transport in the normotensive adult under conditions of "moderate" sodium surfeit. Endogenous dopamine probably does not play a role in the normal renal blood flow regulation but may be important under "stressful" conditions(44). The role and presence of renal DA_2 receptors on renal function remains to be determined.

The mesenteric circulation

Although the effects of graded dosages of dopamine on the renal vascular bed have been well studied, few have examined the effects of dopamine on the mesenteric vascular bed. Pawlik et al(45) found that vasoconstrictor dosages of dopamine, infused into the dog mesenteric vascular bed, decreased oxygen uptake by intestinal tissue. As in the renal vasculature dopamine can affect different types of receptors in the mesenteric circulation, specifically Alpha, Beta-2 and dopamine receptors. The overall effect of dopamine on the mesenteric blood vessels is the algebraic sum of each receptor's effect. Giraud(46) demonstrated with

vasodilatory low dosages of dopamine that intestinal blood flow increased with diminished delivery of oxygen to intestinal tissue as measured using jejunal I^{125} absorption. The dopamine mediated decrease in mucosal flow was abolished by Alpha blockade.

Segal (42) , in a adult porcine model, demonstrated that low dosages of dopamine did not enhance bowel blood flow during hemorrhagic shock but was associated with an earlier onset of gut ischemia. Exacerbation of gut ischemia with dopamine use during progressive hemorrhage was accompanied by a decreased ability of the gut to extract oxygen.

This suggests that Alpha and dopaminergic receptors may be differentially located in mesenteric vasculature; dopamine effect may predominate in the SMA macrovasculature with Alpha effect predominant in mucosal microvasculature. Further autoradiographic localization studies are needed to further evaluate relative receptor densities. The knowledge of the effects of various drugs and naturally occurring compounds on resistance and exchange vessels of the intestine is slowly accumulating. However, the microvascular arterioles, precapillary sphincters, and venules do not necessarily mimic the behavior of their larger parent vessels.

Kullmann and Wassermann(48) emphasize a species and regional difference of dopaminergic vasodilatation in the intestine. In anaesthetized adult rabbits a dopamine mediated flow increase was strongest in the upper part of the GI tract (celiac > SMA). In contrast, in anaesthetized adult cats, regional blood flow rose particularly strongly in the distal part of the intestine (IMA > SMA). There is an apparent differing regional distribution of DA receptors in the splanchnic region of cats and rabbits. This regional distribution of vasodilatation in the bowel vasculature has not been studied in other species.

CHAPTER 5

DOPAMINERGIC EFFECTS IN THE NEWBORN MODEL

The literature suggests a different dopamine dose/response in newborns. Specifically, intravenous administration of dopamine may not always increase renal or mesenteric blood flow or decrease renal or mesenteric vascular resistance in young animals or young humans. Besides the different response to dopamine in newborns there have been some adverse physiologic changes associated with dopamine. First, the role of dopamine as a reliable pressor in the newborn can be questioned. David Driscoll(8), using a neonatal canine model questioned the vasopressor value of dopamine. He used isolated perfused puppy ventricles and atrial strips to demonstrate that the puppy ventricle becomes increasingly responsive to the inotropic effect of dopamine with increasing age. The inotropic effect of dopamine is a direct (minor) action on $Beta_1$ receptors in the myocardium and more importantly, a indirect (major) action mediated by the release of norepinephrine (tyramine-like effect) from sympathetic nerve endings. Driscoll hypothesized that, because the sympathetic innervation of the ventricular myocardium is incomplete at birth, many species have reduced ventricular stores of norepinephrine. The ventricular myocardial tissue of dogs, rabbits and rats achieves the adult complement of sympathetic nerve endings at approximately 3 weeks of age; whereas, the swine and the lamb receive their full complement of sympathetic nerve endings at about 5 days of age(8). It follows that a

decreased responsiveness of the neonatal myocardial tissue to agents that act in part by releasing norepinephrine is expected. Indeed, Driscoll found reduced ventricular isometric contraction in the 0 - 7 day old puppies compared with 21 day old puppies. Driscoll subsequently performed *in vivo* studies with anesthetized puppies(49), monitoring systemic cardiovascular effects of dopamine as well as the effect on the renal vascular resistance. The response of cardiac output to dopamine was less marked in the very young puppies (less than 10 days old) compared to adult dogs but there was an increase in systemic BP and heart rate. This suggests that the cardiovascular system of the puppies was responsive to dopamine but less than that seen in adult dogs. Driscoll found no change in the renal vascular resistance but saw an increase in renal blood flow which likely reflected changes in systemic blood pressure and cardiac output rather than a selective reduction of renal vascular resistance.

Dennis Vane(50), in an acutely instrumented and anesthetized piglet model, with piglets aged 3 - 4 weeks demonstrated that dopamine had no significant effect on the cardiac index compared to controls using dosages from 5 - 50 μg/kg/min. A slight, but statistically insignificant rise in cardiac index, pulse rate, and systemic BP was noted at higher infusion rates. Vane demonstrated that dopamine infused at 10 μg/kg/min did <u>not</u> selectively increase blood flow to the kidney but instead caused renal vasoconstriction. Vane further suggests that renal autoregulation at reduced perfusion pressures (80 mmHg and 50 mmHg) was impaired by the dopamine infusion; the vasoconstriction effect noted was likely mediated by the stimulation of Alpha adrenergic receptors. The author concluded that dopamine's interference with autoregulation may be harmful to the intact kidney in hypotensive states.

Nakamura(51), in newborn lambs, did not see an increase in mean renal blood flow at any given dosage of dopamine (1 - 16 μg/kg/min in fetuses, 2 - 32 μg/kg/min in newborns) until dopamine was infused during Alpha and Beta blockade. Cis-flupentixol, an older dopamine receptor antagonist ($DA_1 > DA_2$), was able to inhibit vasodilatation. Finally, Jaton(52), in newborn rabbits, did not see selective renal vasodilatation with low dosage (4 μg/kg/min) dopamine and reported an increase in renal vascular resistance at an infusion of 10 μg/kg/min of dopamine.

A series of experiments in a variety of species including newborn lambs, puppies, piglets, and rabbits have thus failed to show selective renal vasodilatation with dopamine infusions. In contrast, most reports demonstrated renal vasoconstriction. Evidence suggests that peripheral dopamine receptors are likely not functional in the renal vasculature of neonatal animals, but the majority of these experiments were carried out on acutely instrumented animals with the confounding effects of anesthesia, surgical stress, and positive pressure ventilation.

A chronically instrumented model was needed to validate the results. Anesthetic agents such as ketamine, pentobarbital and halothane have been associated with significant cardiovascular effects in animal models, including decreased cardiac output and increased total peripheral resistance(53). Ketamine and halothane decrease heart rate; pentobarbital increases and halothane decreases systemic BP in piglets(53). Another significant problem with acutely instrumented models is that surgical manipulation of blood vessels produce transient vasoconstriction followed by reactive hyperemia in the organ supplied by the vasculature.

A chronically instrumented model was set up by Debra Fiser et al(54), who instrumented 1 - 2 month old piglets and then allowed them to recover 3

days from anesthesia. She found that dopamine increased cardiac output secondary to an observed increase in heart rate. No significant effect of dopamine on systemic arterial pressure was observed. Dopamine did not alter renal artery blood flow or renal vascular resistance (RVR) in the awake 1 - 2 month old piglet.

Experiments were designed to compare different age groups of animals. Gootman et al(55) found that RVR increased significantly after dopamine bolus injection in newborn piglets; RVR decreased at 2 μg/kg/min in 2 month old piglets only. With a dopamine infusion, Gootman(56) observed that RVR decreased in the newborn piglets at 2 μg/kg/min but increased at 20 μg/kg/min in the newborn and 2 week old group. The discrepancy in these two low dosage results could be explained by the confounding vasodilatory effect of halothane in the second experiment. Gootman also investigated the mesenteric circulation(56); in this study, he observed a decrease in the newborn mesenteric vascular resistance of the 2 week and 2 month old piglets at dosages of 2, 5, and 10 μg/kg/min. Based on these observations Gootman postulated that dopaminergic receptors may be functionally mature in the mesenteric circulation but not in the renal vasculature, although his data does not demonstrate a statistically significant difference in all age groups. Gootman, however, introduced the notion that dopamine receptor postnatal maturity may be organ specific and follow a different time course for different regional circulations.

The literature is sparse regarding the effect of dopamine infusions on bowel. Timothy Feltes et al(57) reported that in newborn (1 week) chronically instrumented lambs dopamine infused at dosages of 5, 20, 80 and 160 μg/kg/min did not selectively dilate any of the vascular beds that they tested (brain, heart, gut, liver, and kidney), and at higher rates of

infusion dopamine actually impaired blood flow to the gut and kidney. No change in organ blood flow was observed at dosages of dopamine less than 80 μg/kg/min; at 80 and 160 μg/kg/min brain and myocardial flow increased while gut and renal flow decreased. Feltes et al found that dopamine at 20 μg/kg/min was uniformly associated with increased cardiac output. Cardiac output is a function of heart rate, preload, afterload and myocardial contractility and since heart rate, preload and peripheral vascular resistance did not change at this dosage, it was assumed that myocardial contractility increased. In higher dosages (80, 160 μg/kg/min) cardiac output decreased and was postulated to be the result of an inotropic:afterload mismatch. Feltes et al concluded that dopamine does not appear to have any specific vasodilatory effect on the organ beds tested and reassured the reader thus, that in conventionally used dosages, the absolute blood flow to the gut and kidneys was not compromised. Since dopamine has been shown to decrease the oxygen extraction of the gut mucosa(45, 46, 47), then even in the absence of macrovascular changes, there is possible concern regarding dopamine related mucosal ischemia.

Other investigators have recently explored dopamine receptor physiology in the systemic circulation and in other regional vascular beds. Polak and Drummond(32), using DA_1 receptor manipulations with fenoldopam (DA_1 agonist) and SCH23390 (DA_1 antagonist) in 7 - 14 day old chronically instrumented lambs demonstrated functional receptors in the pulmonary and systemic circulations. Fenoldopam infusion was associated with an increase in mean pulmonary artery pressure but no change in pulmonary vascular resistance.

In comparing animal models it is important to consider species differences; the general hemodynamics of the piglet and the lamb are

significantly different(50, 57). The newborn lamb is larger than the piglet and therefore has a larger cardiac output and increased resting hemodynamic parameters. The study of dopamine in the swine model is particularly important because the cardiovascular physiology of the swine very closely mimics that of humans.

Dopamine may also produce other theoretical adverse effects in the newborn(58) including: a direct inhibitory effect on carotid body chemoreceptor activity. Dopamine is present in the carotid body of various animals and man. Welsh(59), noted that dopamine depressed minute ventilation during well-oxygenated and anoxic conditions. Supporting previous evidence in cats(60) of a direct inhibitory effect on carotid body chemoreceptor activity. Dopamine may have a detrimental effect on the pulmonary circulation by increasing pulmonary vascular resistance in normoxic or hypoxic dogs(61).

Finally, in newborn humans the literature regarding the effect of dopamine on the systemic, renal and mesenteric vasculature is inadequate, incomplete and occasionally contradictory. There is no good evidence or study that suggests functional renal vasodilatation with low dosage dopamine and no published studies looking at the effect of dopamine on human newborn mesenteric vasculature.

Girardin(62), examined the effect of low dosage dopamine on renal function in post-operative pediatric cardiac patients. Using PAH (para-aminohippurate) and inulin clearance to measure glomerular filtration rate and effective renal plasma flow. Low dosage dopamine (2.5 μg/kg/min) was found to have no effect on cardiac function with a moderate but non-significant effect on renal function in children less than 5 years of age. A

significant increase in renal plasma flow was found, however, in children older than 5 years.

Seri et al(63), administered low dosages of dopamine to preterm human infants who were less than four days old. Using indirect methods such as creatinine clearance, sodium excretion and urine volume to estimate changes in renal blood flow, he concluded on the basis of increased urine volume at 2 and 4 μg/kg/min of dopamine and increased creatinine clearance at 4 μg/kg/min of dopamine that there is indirect evidence for the existence of the direct vasodilatory actions of dopamine in the human preterm neonate. Although Seri's methodology seems reasonable in assessing the renal tubular function, many assumptions are made in data interpretation: indirect measurement of tubular function does not necessarily reflect true change in renal blood flow; in fact, renal natriuretic, kaliuretic and diuretic tubular function appears quite separate from the vasodilatory effect of dopamine(44). Changes in electrolyte excretion can also occur in the absence of changes in renal blood flow or glomerular filtration rate(44). The ability to control the confounding effects of routine supportive care of the newborns in order to detect an independent change in renal blood flow is a difficult if not impossible task. These methodological difficulties lead to questions regarding the investigators' conclusions about the effect of dopamine on renal blood flow. It is technically difficult to directly measure blood flow to vital organs in the newborn human, and animal models provide the next best approximation of human newborn physiology.

The central hemodynamic effects of dopamine in the human newborn are also controversial. The stroke volume of a newborn infant has a limited capacity to increase because of diminished ventricular compliance(64). With a relatively fixed stroke volume, cardiac output is heart rate dependent. Roze et al (65) evaluated ventricular output and blood pressure response in the hypotensive newborn infant to an infusion of dopamine and dobutamine and found that dopamine was more effective than dobutamine in raising blood pressure. Dopamine, however, decreased left ventricular output, as measured by Doppler echocardiography. The clinical literature also documents variable blood pressure responses with dopamine infusions(66,67). There may be an age related maturation of alpha receptors and, consequently, blood pressure response to dopamine. One can conclude that the effect of dopamine on systemic blood pressure in the newborn is neither reliable or predictable.

CHAPTER 6

SUMMARY

Dopamine infusion is associated with a different dose/response in newborn models. The known receptors evidently mature at different rates, Vapaavouri et al(68) demonstrated that Beta-receptor maturation lags behind Alpha-receptor development in fetal lambs and maturation of Beta receptors is less complete at birth than at 2 to 21 days of age. Receptor maturation likely starts prenatally, continues postnatally and applies to all adrenergic receptors including dopaminergic receptors. The most mature adrenergic vascular receptor at birth appears to be the Alpha type (fully mature) followed by Beta and dopaminergic receptors which require significant postnatal development. This hypothesis is supported strongly by Gootman et al (55, 56). It appears that the first few postnatal weeks for the full term animal is likely the crucial maturation period for the dopamine receptor and may be consistent across species barriers(49, 50, 51, 52) but variable from organ to organ within the same species(48, 51, 57).

In most species the renal vasodilatory and natriuretic effects of dopamine increase with age. From work with the kidney, the most studied organ for dopamine receptor studies, one can only conjecture regarding dopamine receptor studies in other organs such as the gut, brain and heart. From recent studies with specific radioligand probes attached to DA_1 antagonists and agonists, it seems that receptor density is not responsible since this does not change or decreases with maturation(17, 44, 69). Renal cortical homogenates were used in these studies. This homogenate

contains both tubular and vascular structures(17). Schmitz et al(70) have reported a good correlation between adrenergic radioligand binding in rat renal tubules membranes and renal vascular effects of adrenergic agents. The efficiency of the receptor/second messenger coupling is evidently key, with a maturational increase in the efficiency of DA_1 receptor-adenylate cyclase coupling(44, 71).

Despite the complex physiology associated with adrenergic receptors a series of animal experiments demonstrated the functional response of the newborn mesenteric and renal vasculatures to dopamine and fenoldopam. In a short term chronically instrumented model using newborn (< 4 days old) swine the measurement of the macrovascular dynamics of the bowel and kidney were recorded in a conscious, spontaneously breathing, relatively "normal" animal. This model allowed recording of the cardiovascular changes without the confounding effects of anesthesia, positive pressure ventilation, and surgical stress.

CHAPTER 7

MATERIALS AND METHODS

Animal model

Mammalian models for in vivo studies are preferred because the results obtained are often applied to human physiology. The use of a model for study is based on one or more of the following criteria: physiological similarity to humans, model availability, historical usage, practicality, and availability of natural occurring disease in animal (if applicable).

Often there is more than one "best" model and the choice may be one of practicality based on: cost, ease of obtaining or maintaining an animal, diet necessary for a specific animal, and handling problems.

From a teleological viewpoint, no animal models are identical to the human, with the possible exception of primates. For the study of developmental physiology, the swine is an excellent model. The pig has some unique advantages over other animals as an experimental model. The hematology, biochemistry, endocrinology, stress response, growth in body weight, control of blood pressure, heart rate, gastrointestinal physiology and regional blood flow all appear to be similar in swine and humans (72, 73, 74, 75). In the renal circulation the similarities of the swine and humans are most related. The completion of nephrogenesis is at 32 - 34 weeks of gestation in man, whereas, it is the third post-natal week in swine. Glomerular filtration rate is 75 ml/min/m^2 in man and 72 ml/min/m^2 in the swine. The maturational increase in proximal convoluted tubule size is 10 - 15 fold in man and 10 fold in the swine. The maximum concentrating capacity of the kidney in man is 1400 mosm/kg; whereas it is 1080 mosm/kg

in the swine. The systemic plasma electrolyte concentrations of the pig are as follows: Na^+=142.5 mEq/L, K^+= 5.6 mEq/L, Cl^-= 100 mEq/L, CO_2=22.5 mEq/L, BUN = 20 mg/dL and creatinine = 1.6 mg/dL(84). These values are all very similar to those in man. Animal models are the best experimental approach to the organ system responses, or interorgan system interactions, of drug studies.

Two groups of piglets of mixed western breed of either gender were obtained on the first or second day of life for instrumentation. In group one (Dopamine), 16 piglets were instrumented in order to obtain 15 mesenteric recordings and 10 renal recordings. In group two (fenoldopam), 14 piglets were instrumented in order to obtain 14 mesenteric recordings and 10 renal recordings. Mesenteric and renal vascular response to dopamine and fenoldopam (a selective DA_1 agonist) were assessed. Fenoldopam was used as a pharmacological probe for DA_1 receptors because dopamine is known to cross-react with alpha and beta receptors at higher dosages. There was no mortality in either group and only one piglet suffered an infarction of the left kidney and was excluded. Migration of the renal probe accounted for the increased number of piglets instrumented to achieve ten in each group.

Surgical procedure

In each animal anesthesia was induced with 5% halothane by mask, this was subsequently reduced to 1 - 2% maintenance and animals breathed spontaneously. Heart rate, arterial oxygen saturation (Nellcor, pulse oximeter, Hayward, California) and rectal temperature (Mon-a-therm, St. Louis, MO.) were monitored continuously. Through a sterile neck incision the left external jugular vein was cannulated with a double lumen

catheter (Arrow, 4F pediatric two-lumen Reading, Pennsylvania). The left common carotid artery was catheterized with a single lumen catheter (Argyle, 5F umbilical vessel catheter Sherwood medical, St. Louis, MO). Both catheters were flushed with heparinized saline, capped and brought out of the neck posteriorly. The neck incision was closed; animals were positioned right side down and a subsequent left flank incision along the lower border of the last rib allowed access to the retroperitoneum. The left kidney was retracted medially and the left renal artery identified. A 2 mm Transonic transit time ultrasound flow probe (Implantable Perivascular Flow probes - Transonic Systems Inc. Ithaca, New York) was placed around the renal artery. Above the renal artery the root of the superior mesenteric artery (SMA) was identified as the next large ventral branch off the aorta. A 3mm Transonic transit time ultrasound flow probe was placed around the SMA (Appendix 4). A suture or fibrin glue (Immuno, Canada Toronto, Ontario) was used to fix the probes in position. The flow probe connectors were tunneled subcutaneously, brought out the posterior neck incision and placed in a protective pouch with the ends of the vascular catheters. After closure of the flank incision, halothane was discontinued and the animal allowed to wake. The total operative time for each animal approximated 60 minutes. Animals were returned to their cages, their activity was unrestricted and they were allowed to feed on a porcine milk substitute *ad libitum.* Vascular catheters were flushed as needed with heparinized saline. The presence of normal feeding, stooling, urination and post-operative weight gain was monitored as an indication of piglet well being. A room temperature of 26 degrees Celsius was maintained and an extra infra-red heating lamp was positioned 4 feet above the floor of the cage. Rectal acetaminophen (10 mg/kg) was used as necessary for post-operative analgesia.

Experimental procedure

Two days post-operatively each animal was returned to the laboratory after a 3 hour fast (Appendix 5). Each conscious non-sedated piglet was placed in a modified cat box (CDMV inc. St. Hyacinthe, Quebec), this minimal restraint environment allowed the animal to rest comfortably. One limb of the double lumen catheter in the external jugular vein was attached to an infusion of normal saline while the second limb was attached to a dopamine or fenoldopam infusion. Dopamine and fenoldopam solutions were prepared daily in normal saline. Dopamine or fenoldopam was infused with a micro-flo-guard 8500 rotating disk volumetric infusion pump (Travenol Labs Inc. Deerfield, Illinois) and normal saline was infused with an Imed 965 micro volumetric infusion pump (Imed corporation San Diego, California). The foregoing allowed the total intravenous infusion rate to be kept constant at 4cc/kg/hour throughout each experiment. The carotid arterial line was connected to a pressure transducer and infusion system which was amplified by a HP 78342A clinical monitor (Hewlett Packard) and the transonic SMA flow probe and renal flow probe were connected to a flow meter (T206 dual channel small animal blood flow meter - Transonic systems inc. Ithaca, New York). Arterial oxygen saturation, heart rate, mean arterial pressure, SMA flow and renal arterial flow were recorded continuously, with analog data digitized by an analog to digital converter (Datatranslation, model DT2801A Mississauga, Ontario) and acquired using a 486 / 25 megahertz personal computer running the ASYST computerized software data acquisition program(76). Central venous pressure was measured intermittently and recorded manually.

After baseline measurements in the first group of ten piglets dopamine was infused intravenously in doses of 2, 4, 8, 16, and 32 µg/kg/min in random order. In the second group of ten piglets fenoldopam was infused in dosages of 1, 5, 10, 25, 50, 100 µg/kg/min after baseline measurements. Data were recorded for 10 minutes after a 10 minute infusion period; subsequently the infusion was discontinued and a further 15 minutes period allowed clearance of drug (half-lives of a few minutes with both drugs) (1, 36). Between each administration of dopamine or fenoldopam, a separate baseline was determined over an interval of 5 minutes (Appendix 6) and mean baseline values were compared to mean values post-infusion, allowing each animal to act as its own control. No residual effect between dosages of fenoldopam or dopamine was observed as baseline values were comparable.

Calculated values

All pressures and flows were directly measured as described. The mesenteric and renal vascular resistances were calculated using Ohm's law for the circulation(77).

Ohm's Law for the circulation

$$\text{Resistance} = \frac{\text{delta pressure}}{Q\ (\text{flow})} \quad \text{or;} \quad \text{Mesenteric/renal resistance} = \frac{\text{MAP - CVP}}{\text{mesenteric/renal flow}}$$

MAP = Mean arterial pressure

CVP = Central venous pressure

Statistical methods

Sample size /statistical power

The sample size required in each group was calculated using the following predetermined statistical parameters: type 1 error rate of 0.05 (alpha), type 2 error rate of 0.20 (beta) and an estimated variability of 25% in data, expressed as standard deviation. This provided adequate statistical power and required a sample size of 10 in each group in order to detect a 35% change in blood flow(78). A 35% change in blood flow was a conservative estimate because dopamine is known to increase renal blood flow 50% in adult humans(2) and fenoldopam reported to increase renal blood flow 86% in adult humans(38).

Data analysis

The mean percentage change from baseline values of all animals at each dosage of drug were compared to the values post-infusion using a one way ANOVA (repeated measures analysis of variance) with the help of Sigmastat software (Jandel scientific, San Rafael, California). The Student-Newman-Keuls multiple comparisons procedure was used to compare differences among dose responses. Both groups of ten piglets each were analyzed individually. A significance level of < 0.05 was considered acceptable. All results are expressed as mean +/- standard deviation.

CHAPTER 8

RESULTS

The results of each of the two groups are summarized in table form for dopamine (Table 1) and fenoldopam infusion (Table 2).

GROUP 1 DOPAMINE

Effects of dopamine on superior mesenteric artery blood flow (figure 1)

At lower dosages of dopamine (2, 4, and 8 μg/kg/min) there was no significant effect on superior mesenteric artery blood flow. In contrast, at higher dosages (16 and 32 μg/kg/min) there was an increase in superior mesenteric artery blood flow, this was significant at 32 μg/kg/min ($p < 0.05$).

Effects of dopamine on superior mesenteric artery vascular resistance (figure 2)

At all dosages studied there was no significant effect of dopamine on mesenteric vascular resistance.

Effects of dopamine on renal artery blood flow (figure 3)

No significant effect of dopamine on renal artery blood flow occurred at dosages of 2, 4, 8 and 16 μg/kg/min. At 32 μg kg/min a significant decrease in renal artery blood flow occurred ($p < 0.05$).

Effects of dopamine on renal artery vascular resistance (figure 4)

No significant effect of dopamine on renal artery vascular resistance occurred at dosages of 2, 4, 8 and 16 μg/kg/min. At 32 μg kg/min a significant increase in renal artery vascular resistance occurred ($p < 0.05$).

Effects of dopamine on systemic mean arterial blood pressure (figure 5)

No significant effect of dopamine on systemic mean arterial blood pressure occurred at any of the dosages studied.

GROUP 2 FENOLDOPAM

Effects of fenoldopam on superior mesenteric artery blood flow (figure 6)

A significant increase in superior mesenteric artery blood flow occurred at dosages of 5, 10, 25, 50 and 100 μg/kg/min of fenoldopam ($p < 0.05$). No significant change occurred at the lowest dosage of 1 μg/kg/min.

Effect of fenoldopam on superior mesenteric artery resistance (figure 7)

A significant decrease in superior mesenteric artery resistance occurred at dosages of 5, 10, 25, 50 and 100 μg/kg/min of fenoldopam ($p < 0.05$). No significant change occurred at the lowest dosage of 1 μg/kg/min.

Effects of fenoldopam on renal artery blood flow (figure 8)

No significant effect of fenoldopam on renal artery blood flow occurred at any of the dosages studied.

Effects of fenoldopam on renal artery vascular resistance (figure 9)

No significant effect of fenoldopam on renal artery vascular resistance occurred at any of the dosages studied.

Effects of fenoldopam on mean systemic arterial pressure (figure 10)

Fenoldopam decreased mean systemic arterial blood pressure at all dosages studied, this decrease was significant at 5, 10, and 100 μg/kg/min ($p < 0.05$).

CHAPTER 9

DISCUSSION

There is little information regarding the ontogeny of catecholamine receptors in the mesenteric or renal vasculature of neonatal mammals and no information available for the newborn human. The effects of dopamine infusion and dose-response relationships cannot be predicted from adult models, but require investigation.

Vasodilatation of the superior mesenteric artery (SMA) and renal artery was not observed with low dosage (2 - 4 μg/kg/min) dopamine in newborn (< 4 days) piglets. Contrary to popular belief, dopamine is not a renal or mesenteric vasodilator in the clinically-used low dosage range, in marked contrast to adult animal models in which dopamine has been shown to have a vasodilatory effect (2, 3, 5, 79). This likely reflects a biologic functional immaturity of the DA_1 dopaminergic receptor system and the subsequent coupled physiological response of vascular smooth muscle in superior mesenteric and renal arteries. This suggests that there is little to recommend the use of dopamine at "renal dosages" in the newborn. Low dose dopamine has frequently been used in an attempt to maintain renal and bowel perfusion during inotropic therapy in the critically ill; but, this and the work of others suggests that such effort is unlikely to be successful.

The kidney has become the prototype organ for dopamine receptor studies and a number of authors have reported a lack of renal vascular vasodilatation with low-dosage dopamine in young puppies(49), newborn

lambs(51), rabbits(52) and acutely instrumented piglets(50, 55). Most reports have, in fact, documented an increase in renal vascular resistance.
In further support Feltes et al(57) found, in lambs, no selective vasodilatation in the vascular beds of brain, heart, gut, liver and kidney in response to low dosage dopamine; at dosages of 80 and 160 μg/kg/min dopamine actually impaired blood flow to the gut and kidney.

The vasoconstriction in the renal vasculature with the highest dosage of dopamine likely represents the stimulation of relatively mature alpha receptors. Once a sufficient dosage of dopamine was infused to obtain an increase in blood pressure (32 μg/kg/min), a significant decrease in blood flow was observed. Clinically, newborn infants appear to be able to achieve an increase in blood pressure during dopamine infusion at somewhat lower dosages than those required in the piglet(8), suggesting that the alpha effects of dopamine may predominate at lower dosages in the human neonate putting the renal circulation at even greater risk.

Since dopamine cross-reacts with alpha and beta receptors at higher dosages, a further series of ten piglets were administered fenoldopam (DA_1 agonist) as a specific pharmacological probe for DA_1 receptors. Fenoldopam has neither central nervous system dopamine receptor activity nor peripheral DA2 receptor activity(33). Using dosages many times greater than those in adult models, fenoldopam was not able to stimulate a vasodilatory response in the renal artery. This observation further supports the functional immaturity of the DA_1 receptor mechanism in the newborn renal circulation.

In the mesenteric circulation, the increase in SMA blood flow that was observed at 16 and 32 μg/kg/min ($p < 0.05$) has been reported at 20 μg/kg/min by other authors(56, 57), and in part, likely reflects a cross reaction with alpha and beta adrenergic receptors. Since mesenteric vascular resistance did not

significantly change, it is reasonable to assume that the increase in systemic blood pressure was responsible for the observed increase in mesenteric blood flow. The absence of a vasoconstrictory effect at the higher dosages of dopamine (16 and 32 μg/kg/min) suggests there is likely to be an opposing vasodilatory dopaminergic effect which counteracts the constricting effect of the alpha receptors.

Group 2 was needed to determine whether the increased blood flow in the mesenteric circulation with higher dosages of dopamine was a beta-2 or dopaminergic effect. Fenoldopam was used as a specific pharmacological probe for the DA_1 receptors. Superior mesenteric artery vasodilatation occurred with all dosages of fenoldopam studied except the lowest dosage of 1 μg/kg/min. Dopamine in higher dosages (16, 32 μg/kg/min) was associated with an increase in blood flow in the mesenteric circulation, suggesting that dopaminergic receptors may be functional but the vasodilatory mechanism has reduced sensitivity in the mesenteric circulation of the newborn piglet. In order to demonstrate this definitively, the responses observed using fenoldopam would have to be abolished using a DA_1 antagonist. Unfortunately, there is no other comparable direct investigation of the dopaminergic vascular effects in the mesenteric circulation of the newborn animal reported. One can, perhaps, assume that dopamine DA_1 receptors are present in the neonatal mesenteric circulation but require greater concentrations of dopamine in order to activate them.

Besides being a DA_1 receptor agonist, fenoldopam at high dosages has been shown to be an alpha receptor antagonist(82, 83). This hemodynamic effect of fenoldopam is controversial because some investigators suggest that fenoldopam is specific from 0.1 - 100 μg/kg/min(30, 31), while others(39) suggest that it cross-reacts with alpha receptors at much lower dosages. This

possible effect must be considered, however, and may have complicated the pharmacological picture in studying the mesenteric circulation at the highest dosages of fenoldopam. The alpha antagonist effect of fenoldopam likely was not significant as no vasodilatation was observed in the renal circulation of the piglets. Various dosages of fenoldopam have been used in animal models ranging from 0.1 - 10,000 μg/kg/min (30, 31, 32, 33, 34, 35). The dosage range of 1 - 100 μg/kg/min was chosen because fenoldopam has been reported specific in this range(30, 31).

The effect of dopamine on systemic blood pressure was not significant or predictable. This observation has been made by others (49, 50, 54, 55, 56, 57, 66, 67) in both experimental and clinical settings. This observation may possibly be explained by a variable maturation of the alpha receptors which produce vasoconstriction and increase blood pressure. Blood pressure is a function of cardiac output multiplied by peripheral vascular resistance. Although cardiac output was not directly measured, a failure of dopamine to increase cardiac output based on a inotropic - afterload mismatch cannot be ruled out. Driscoll(8), has also hypothesized that the incomplete sympathetic innervation of the neonatal myocardium impairs the ability of dopamine to augment cardiac output.

Fenoldopam decreased blood pressure at all dosages greater than 1 μg/kg/min and this was significant at 5, 10, and 100 μg/kg/min which suggests that certain vascular beds in the newborn piglet have functional DA_1 receptors. The renal vasculature was not demonstrated to be one of these vascular beds. Alternatively, it is possible that alpha receptors were antagonized at the highest dosages of fenoldopam resulting in a decrease in blood pressure. In order to prove that dopaminergic receptors were responsible for the decrease in blood

pressure, the response would have to be abolished by pretreatment of the piglets with a DA_1 antagonist.

This data supports the theory of Gootman and others(55, 56, 80) that in the post-natal period, vascular autonomic receptors mature at different rates(56), with the alpha adrenergic receptor being relatively mature at birth but dopaminergic receptors requiring significant post-natal development.

The macrovascular responses of the renal and mesenteric circulations have been quantified in this study but may not represent the microcirculatory changes. In adult animal models with fully functional DA_1 receptors low dosage dopamine (2 μg/kg/min) has been shown to decrease the oxygen extraction of gut mucosa, and to possibly increase the risk of mucosal ischemia(46, 47); these effects may be mediated by alpha receptors in the microcirculation(45). Future investigation of the pharmacological effects of these drugs on the microcirculation should involve techniques such as intestinal tonometry and renal inulin clearance to estimate the effects on intestinal mucosal blood flow and renal glomerular filtration rate. Inulin clearance is more reliable than para amino hippurate clearance in the newborn because para amino hippurate extraction is known to be incomplete in small children and is therefore a poor indicator of total renal plasma flow(84).

Most investigators have focused on the developmental changes in the cardiovascular system to explain the different response to dopamine in newborns. Another theoretical explanation for the lack of effect observed with dopamine infusion in the newborn may be developmental changes in the pharmacokinetics of the drug(54). Padbury(81), measured the plasma catecholamine levels in a small number of critically ill newborn infants receiving dopamine infusions and reported that plasma dopamine concentrations varied widely with variable cardiovascular response. Other investigators(1, 85, 86)

have also shown that there is significant inter-individual variation among patients receiving dopamine. Goodall and Alton(87) demonstrated that 25% of infused dopamine is used for the synthesis of endogenous norepinephrine stores and subsequently used variable amounts of the infused dopamine to replenish such stores. This varied uptake of dopamine may explain the wide variation seen in patient pharmacokinetics. It the absence of direct data in piglets, it is necessary to speculate about each phase of the pharmacokinetics of dopamine. The absorption of a drug is determined primarily by the route of administration. In this study, both dopamine and fenoldopam were administered by intravenous infusion, this avoided the influence of variable gastrointestinal absorption and first pass liver metabolism. The volume of distribution in the neonate is different with a higher percentage of body weight in the form of water (70 - 75%) than the adult (50 - 60%). The extracellular water is 40% of body weight in the neonate compared with 20% in the adult. Since dopamine is distributed throughout the extracellular water space in order to reach the receptors, the size (volume) of the extracellular water compartment is important in determining the concentration of drug at the receptor sites. The protein binding of dopamine has been reported to be 30% by Banner et al(88); in general the protein binding of drugs is reduced in the neonate, this should increase the concentration of free (unbound) drug in plasma. This theoretical situation should increase the effects of dopamine, however this is not observed. The metabolism of dopamine by monoamine oxidase and catechol-o-methyltransferase occurs both in the periphery and in the liver. The drug metabolizing activity of these enzymes are likely substantially lower in neonates than later in life. The excretion of dopamine and metabolites by the kidney is likely impaired because GFR of a neonate is only 30 - 40% and tubular secretion 20 - 30% of adult values(89). Overall, it seems unlikely that

differences in pharmacokinetics can account for the differences in drug action between this model and more mature animals.

The ontogeny of the other adrenergic receptors have received little attention. In the renal circulation of the developing sheep, in vitro studies of alpha receptor response in isolated segments of renal artery have demonstrated an age related decreased responsiveness of vascular smooth muscle to alpha receptor agonists. The authors(90) speculate that this reflects a decrease in alpha receptor number, affinity or difference in smooth muscle function. An in vivo study(91) in lambs confirmed the previous observations. This study demonstrated a decreased alpha receptor density using radioligand binding techniques. Predominance of vasoconstriction in the renal circulation in early life may be explained by these observations.

In summary, there remain several areas in need of further study. This includes: a better definition of the ontogeny of alpha and beta receptors using pharmacological manipulations with available agonists and antagonists, radioligand binding of selective receptor probes with binding affinity studies, dopaminergic effects in other circulations such as the cerebral and coronary beds with and without dopamine blockers. Studies of the microvascular effects of dopamine using tonometry in the bowel and inulin clearance of the kidney. The distribution, metabolism and excretion of dopamine in both healthy and sick newborns must be studied. Finally, the sub-cellular coupling of dopamine to second messenger and the end organ response of vascular smooth muscle must be better defined using measurements of adenylate cyclase activity in vitro. The preferred animal model for all future newborn dopamine receptor studies is an in vivo preparation using the newborn piglet. This model allows the best approximation of organ and interorgan responses to drugs in the newborn human.

CHAPTER 10

CONCLUSIONS

No protective vascular vasodilatory effect of dopamine was observed in the renal or mesenteric circulations of the piglet. This different dopamine dose/response can be explained by a post-natal maturation or ontogeny of the various adrenergic receptors(55, 56, 80). In the kidney, it has been shown that the receptor density is unchanged with age(17) but that an enhanced biological coupling of receptor with cAMP second messenger accounts for the observed differences between newborns and adults(44). This receptor ontogeny is different for each receptor type and was demonstrated to be organ specific even within the same species. The renal dopamine DA_1 receptors were not stimulated with either dopamine or fenoldopam. In contrast, the mesenteric DA_1 receptors were stimulated with high dosage dopamine and all dosages of fenoldopam greater than 1 μg/kg/min. This demonstrates that dopamine receptor maturation is more advanced in the mesenteric circulation of the four day old piglet.

The blood pressure response of the newborn piglet to dopamine was variable, without significant effect observed. This suggests variable intersubject alpha and possibly beta receptor maturation.

Caution should be exercised when using dopamine in the newborn setting as the effects produced are different than those in adults. Dopamine was not found to protect two vital vascular beds in the piglet, and the risk of bowel mucosal ischemia(46, 47) may raise further concern with the use of dopamine to treat hypotension. In addition, others have reported an inhibitory

effect of dopamine on carotid body chemoreceptor activity(59), and a detrimental increase in pulmonary vascular tone associated with dopamine infusion(61).

The piglet was chosen as the animal model in our experiments since the physiology is similar to the human neonate. Although it is clearly not possible to directly extrapolate from one species to another it makes sense when the general pattern of responses in all mammalian species are consistent, to assume, in the absence of direct human data, that human infants probably respond in a similar way. Dopamine is the most commonly used inotrope in newborns, this practice has been widely adopted based on the effects produced in adults. In consideration of the evidence reported it is recommended that caution be exercised with dopamine infusion and that other catecholamines with possible beneficial effects on the vital organs of the newborn such as epinephrine and dobutamine be investigated.

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TABLE 1 DOPAMINE RESULTS (n=16)

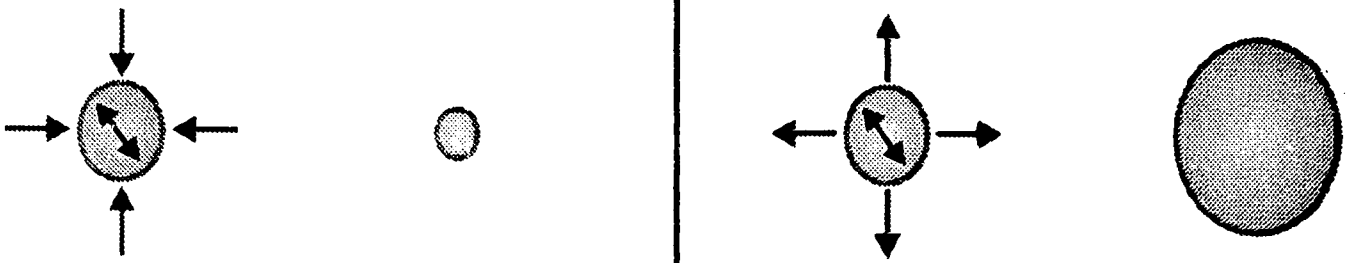
Dop. group1 n=16	*DOSE µg/kg/ min.*	*2*	*4*	*8*	*16*	*32*
B.P. mm/Hg	*mean*	*73.75*	*74.03*	*76.42*	*78.35*	*83.42*
B.P. mm/Hg	*S.D. +/-*	*15.16*	*16.25*	*16.07*	*16.82*	*22.57*
CVP mm/Hg	*mean*	*3.5*	*4.5*	*4.5*	*4.75*	*5.083*
CVP mm/Hg	*S.D. +/-*	*1.624*	*2.316*	*1.382*	*1.603*	*2.151*
SMAf ml/min	*mean*	*86.43*	*86.83*	*92.62*	*92.92*	*93.53*
SMAf ml/min	*S.D. +/-*	*48.4*	*47.98*	*50.46*	*50.06*	*52.03*
RENf ml/min	*mean*	*20.43*	*20.53*	*22.11*	*22.85*	*19.61*
RENf ml/min	*S.D. +/-*	*14.8*	*12.28*	*13.37*	*13.94*	*10.23*
SMAr PRU	*mean*	*1.189*	*1.146*	*1.035*	*1.116*	*1.215*
SMAr PRU	*S.D. +/-*	*0.896*	*0.827*	*0.691*	*0.755*	*0.993*
RENr PRU	*mean*	*5.644*	*5.291*	*5.208*	*4.973*	*5.372*
RENr PRU	*S.D. +/-*	*4.225*	*4.597*	*4.722*	*4.483*	*4.268*
%RENf	*mean*	*-3.156*	*-1.525*	*1.03*	*-4.876*	*-8.581*
%RENf	*S.D. +/-*	*16.48*	*6.021*	*14.25*	*25.68*	*30.15*
%SMAf	*mean*	*-2.698*	*0.069*	*3.549*	*15.04*	*15.58*
%SMAf	*S.D. +/-*	*10.93*	*8.104*	*20.4*	*19.07*	*30.19*
%RENr	*mean*	*3.147*	*1.695*	*5.539*	*20.33*	*38.02*
%RENr	*S.D. +/-*	*18.18*	*10.92*	*26.26*	*54.57*	*59.65*
%SMAr	*mean*	*0.977*	*1.078*	*0.466*	*-8.091*	*1.812*
%SMAr	*S.D. +/-*	*10.37*	*12.09*	*14.41*	*16.9*	*40.73*
weight Kg	*mean*	*1.838*	*1.838*	*1.838*	*1.838*	*1.838*
weight Kg.	*S.D. +/-*	*0.32*	*0.32*	*0.32*	*0.32*	*0.32*
Age days	*mean*	*3.75*	*3.75*	*3.75*	*3.75*	*3.75*
Age days	*S.D. +/-*	*0.683*	*0.683*	*0.683*	*0.683*	*0.683*

TABLE 2 FENOLDOPAM RESULTS (n=14)

Fenold. group 2 n=14	*DOSE µg/kg/ min.*	*1*	*5*	*10*	*25*	*50*	*100*
B.P. mm/Hg	*mean*	*75.73*	*68.59*	*68.86*	*78.61*	*78.42*	*74.71*
B.P. mm/Hg	*S.D. +/-*	*13.55*	*10.18*	*9.105*	*20.95*	*12.54*	*8.751*
CVP mm/Hg	*mean*	*5.75*	*5*	*6.125*	*6*	*6.25*	*6.25*
CVP mm/Hg	*S.D. +/-*	*2.121*	*1.309*	*1.808*	*1.773*	*1.669*	*1.982*
SMAf ml/min	*mean*	*132.5*	*184.6*	*177.3*	*161.4*	*159.2*	*157.5*
SMAf ml/min	*S.D. +/-*	*36.43*	*53.29*	*48.38*	*53.98*	*42.49*	*51.15*
RENf ml/min	*mean*	*28.41*	*30.48*	*26.51*	*21.87*	*25.71*	*27.81*
RENf ml/min	*S.D. +/-*	*5.652*	*3.882*	*5.763*	*2.48*	*2.965*	*6.955*
SMAr PRU	*mean*	*0.567*	*0.365*	*0.38*	*0.503*	*0.48*	*0.474*
SMAr PRU	*S.D. +/-*	*0.203*	*0.108*	*0.125*	*0.227*	*0.131*	*0.15*
RENr PRU	*mean*	*2.552*	*2.136*	*2.468*	*3.558*	*2.898*	*2.624*
RENr PRU	*S.D. +/-*	*0.174*	*0.164*	*0.392*	*1.643*	*0.33*	*0.47*
%RENf	*mean*	*13.99*	*-5.246*	*-8.449*	*-11.87*	*-4.454*	*0.474*
%RENf	*S.D. +/-*	*20.22*	*10.63*	*4.731*	*19.91*	*8.67*	*0.15*
%SMAf	*mean*	*2.155*	*48.16*	*33.92*	*24.57*	*15.18*	*-1.265*
%SMAf	*S.D. +/-*	*26.34*	*26.47*	*19.78*	*32.21*	*13.46*	*8.783*
%RENr	*mean*	*-13.65*	*-4.156*	*-5.967*	*28.97*	*3.525*	*-4.885*
%RENr	*S.D. +/-*	*19.76*	*10.52*	*7.462*	*79.37*	*24.94*	*16.05*
%SMAr	*mean*	*-4.546*	*-38.59*	*-34.41*	*-18.91*	*-16.88*	*-21.34*
%SMAr	*S.D. +/-*	*28.71*	*11.34*	*14.72*	*22.14*	*25.27*	*26.69*
weight Kg	*mean*	*1.74*	*1.74*	*1.74*	*1.74*	*1.74*	*1.74*
weight Kg.	*S.D. +/-*	*0.45*	*0.45*	*0.45*	*0.45*	*0.45*	*1.74*
Age days	*mean*	*4*	*4*	*4*	*4*	*4*	*4*
Age days	*S.D. +/-*	*0*	*0*	*0*	*0*	*0*	*0*

Dopamine Effect on Adrenergic Receptors in Vascular Smooth Muscle

Receptors That Vasoconstrict	Receptors That Vasodilate
α Receptors	DA_1 Receptors β Receptors



Overall response of blood vessel is algebraic.
Sum of each receptor's effect.

Table 3

Criterion	DA_1 Receptor
Prototype	Vascular Smooth Muscle
Function	Vasorelaxation
Cyclase Linkage	Stimulatory
Agonists	Fenoldopam
Antagonists	SCH 23390 & SCH 39166
Location	Pre and Post-synaptic

Table 4

Figure 1

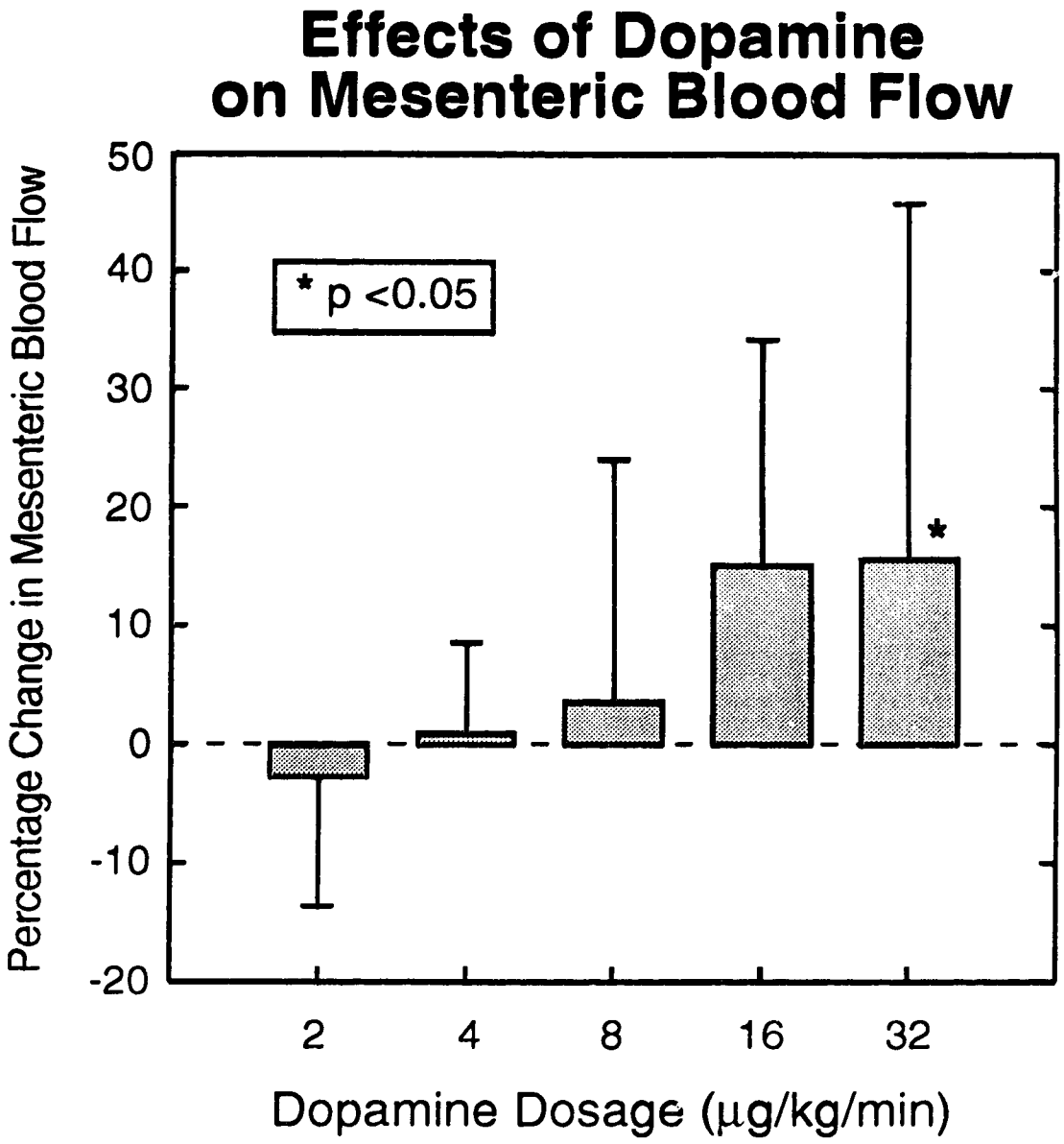

Effects of Dopamine
on Mesenteric Blood Flow
Percentage Change in Mesenteric Blood Flow
50
40
30
20
10
0
-10
-20
* p <0.05
*
2
4
8
16
32
Dopamine Dosage (µg/kg/min)

Figure 2

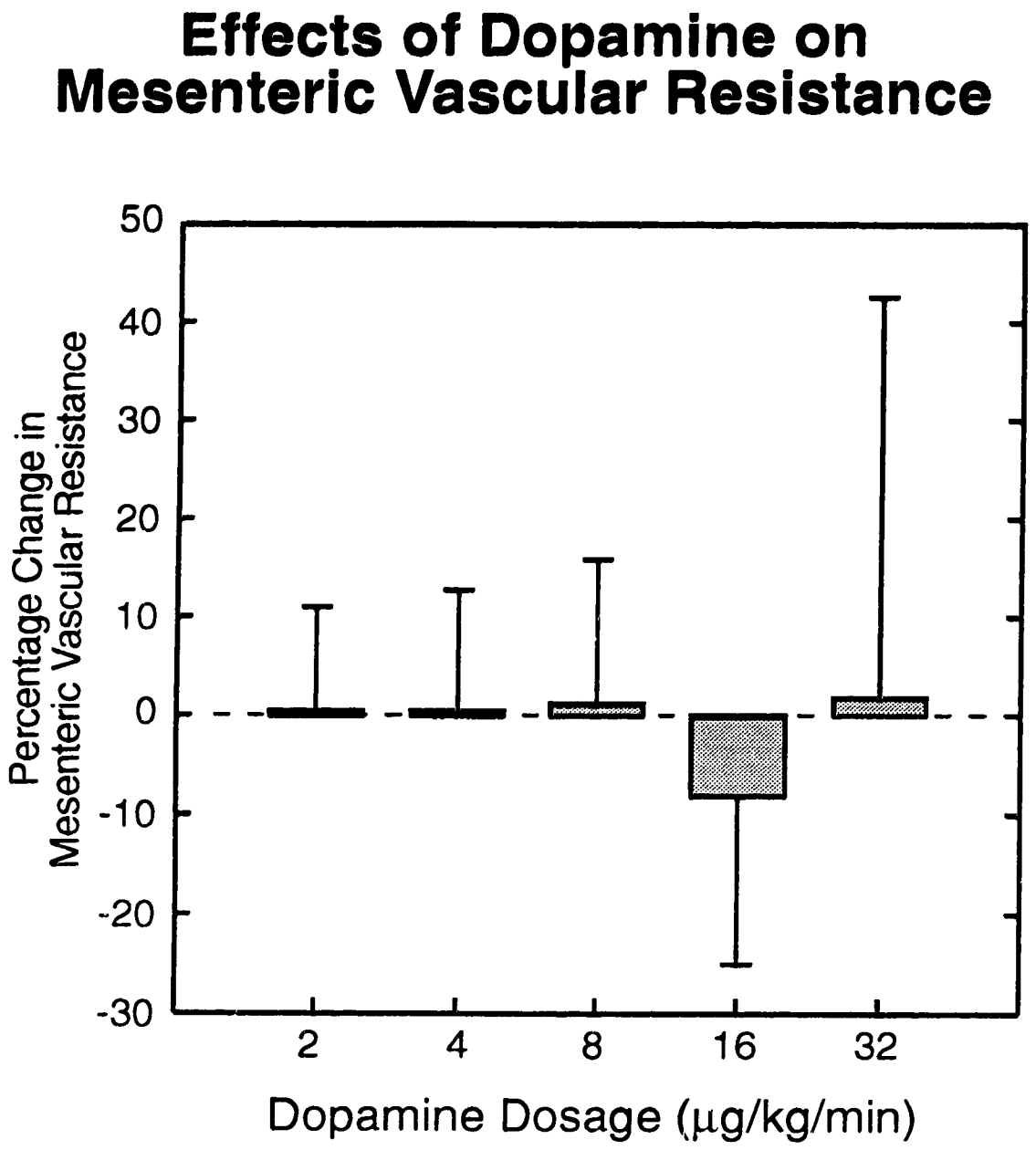

Effects of Dopamine on
Mesenteric Vascular Resistance
Percentage Change in
Mesenteric Vascular Resistance
50
40
30
20
10
0
-10
-20
-30
2
4
8
16
32
Dopamine Dosage (µg/kg/min)

Figure 3

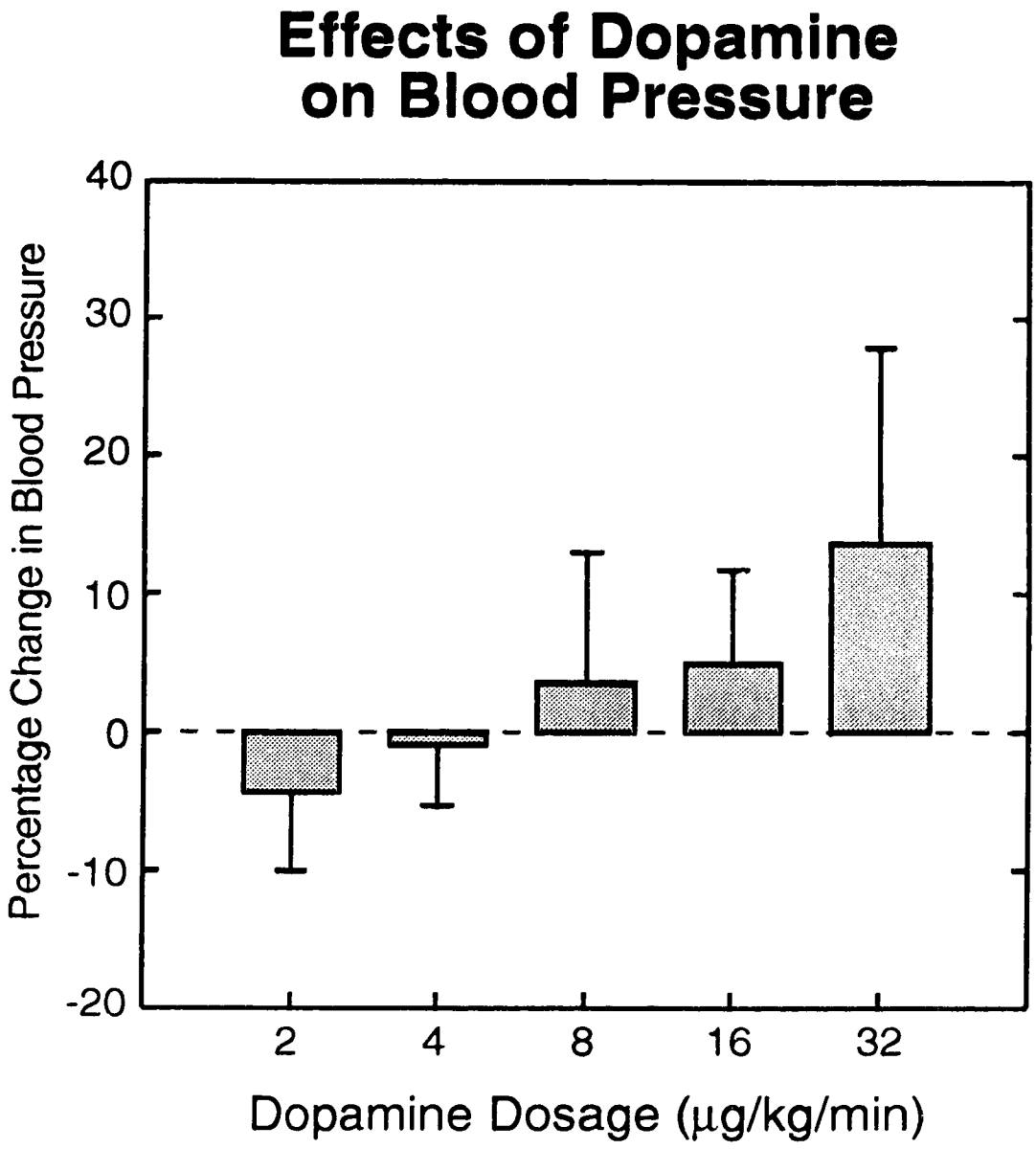

Effects of Dopamine on Blood Pressure
Percentage Change in Blood Pressure
40
30
20
10
0
-10
-20
2
4
8
16
32
Dopamine Dosage (µg/kg/min)

Figure 4

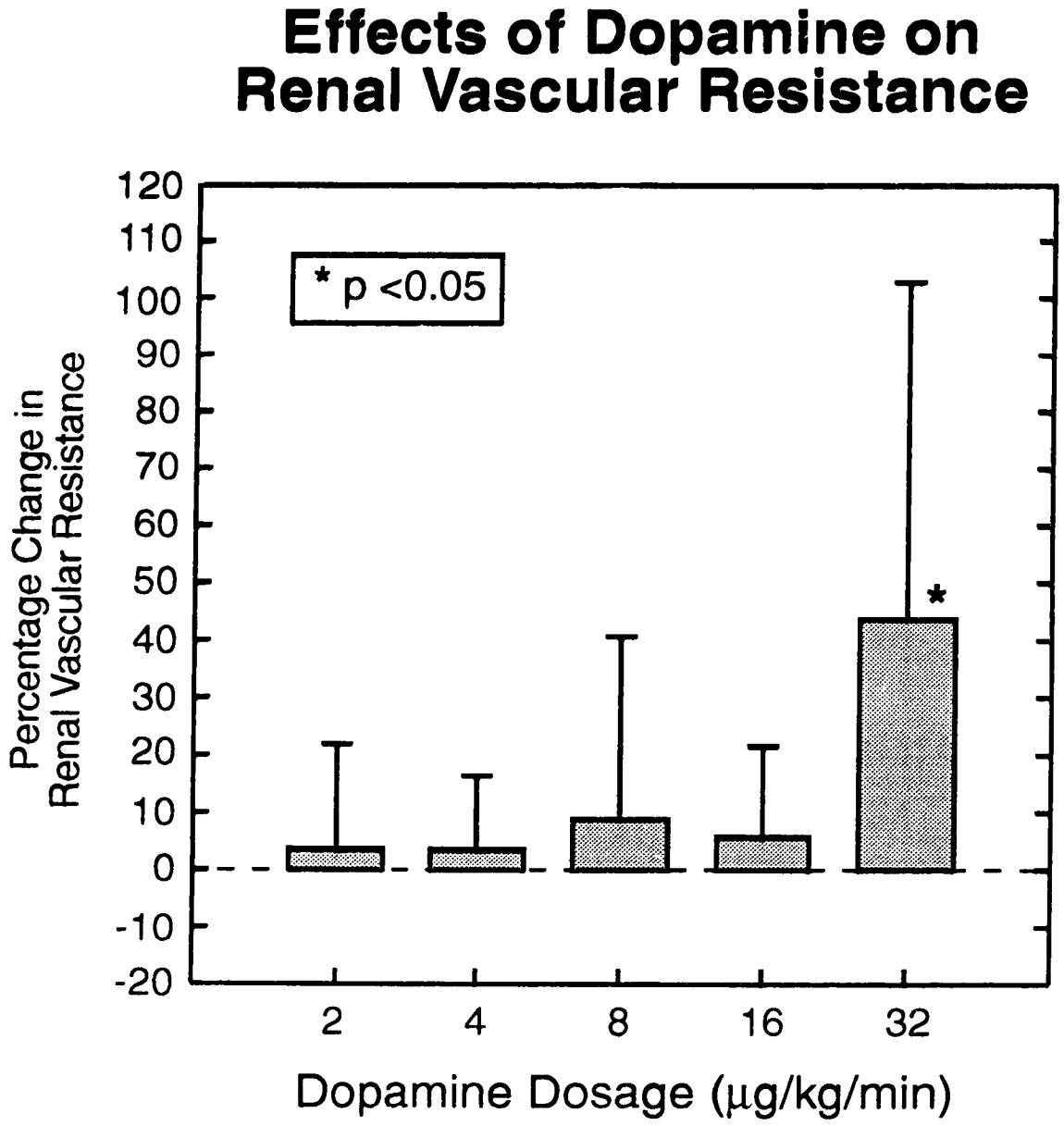

Effects of Dopamine on
Renal Vascular Resistance
Percentage Change in
Renal Vascular Resistance
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
* p <0.05
*
2
4
8
16
32
Dopamine Dosage (µg/kg/min)

Figure 5

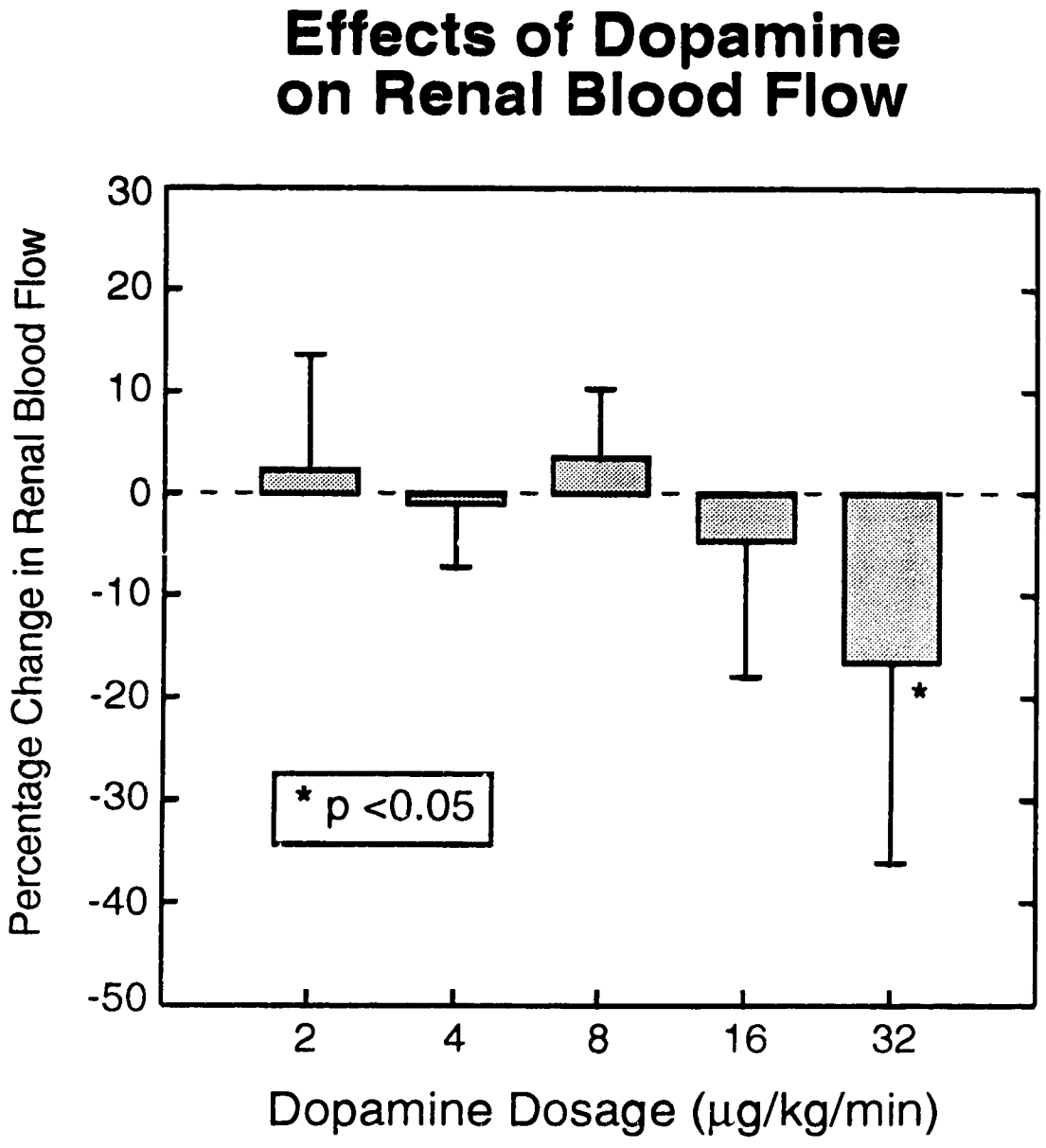

Effects of Dopamine
on Renal Blood Flow
Percentage Change in Renal Blood Flow
30
20
10
0
-10
-20
-30
-40
-50
* p <0.05
*
2
4
8
16
32
Dopamine Dosage (µg/kg/min)

Figure 6

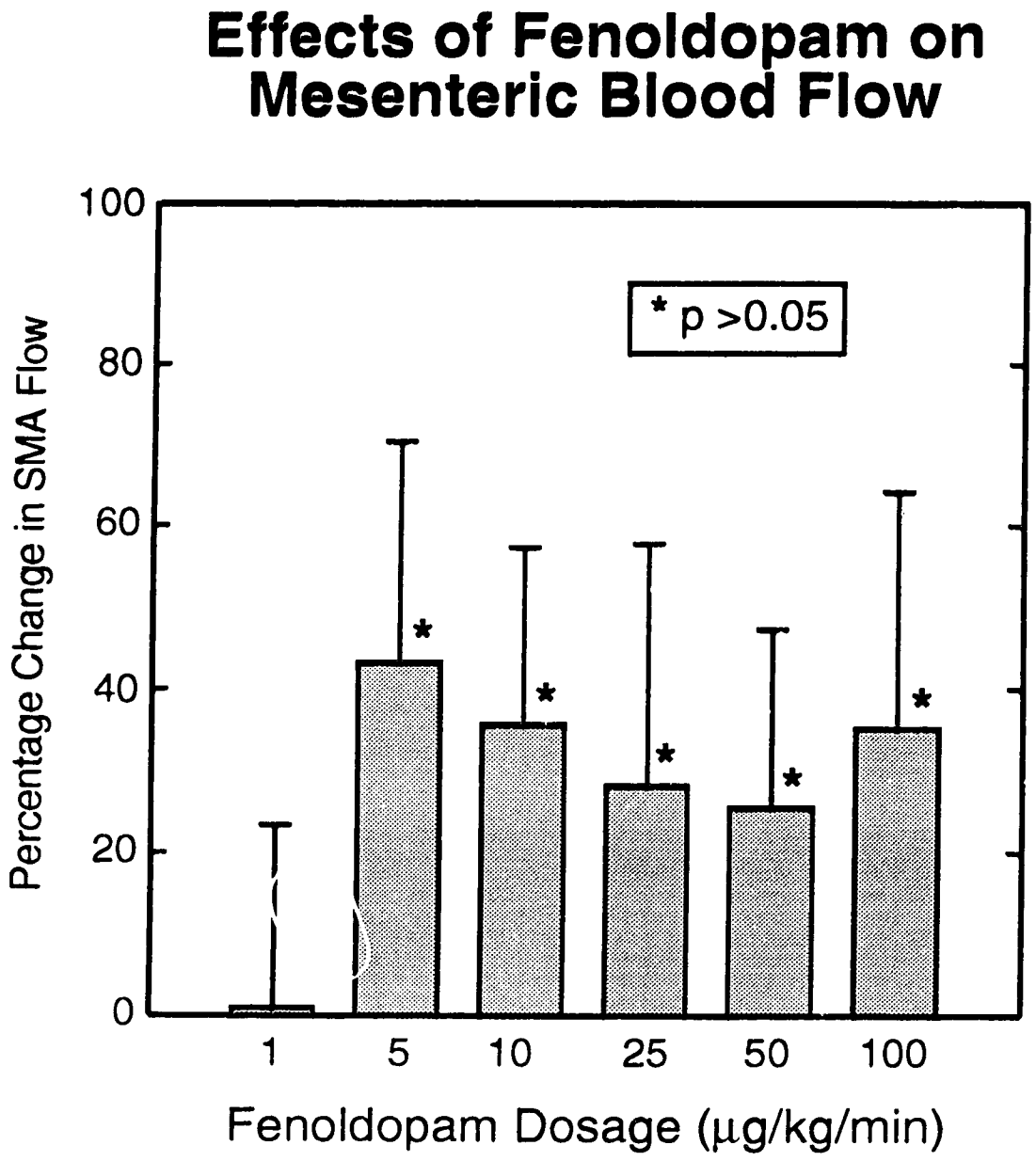

Effects of Fenoldopam on Mesenteric Blood Flow
* p >0.05
Percentage Change in SMA Flow
100
80
60
40
20
0
1
5
10
25
50
100
Fenoldopam Dosage (µg/kg/min)

Figure 7

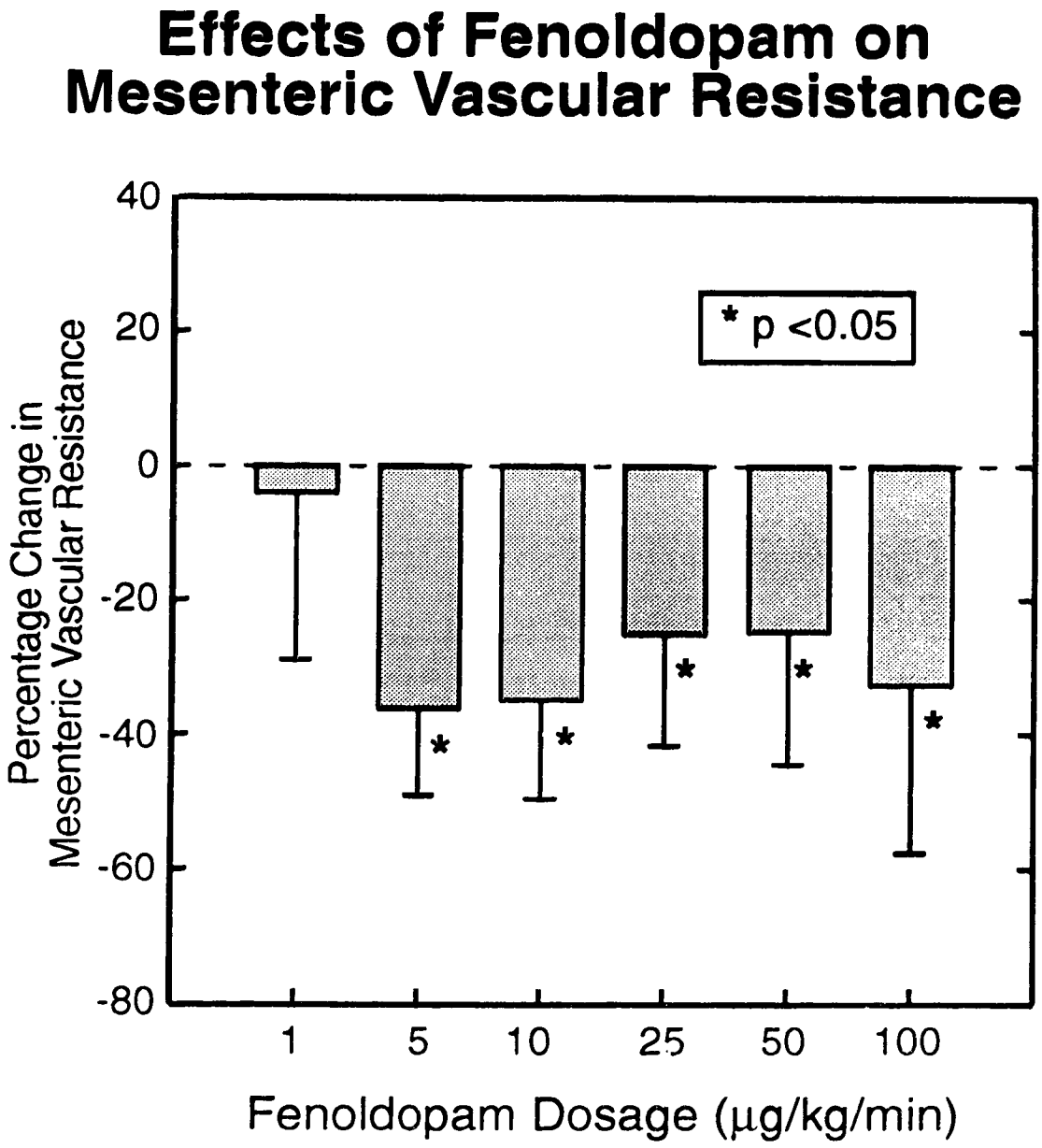

Effects of Fenoldopam on Mesenteric Vascular Resistance
Percentage Change in Mesenteric Vascular Resistance
40
20
0
-20
-40
-60
-80
* p <0.05
*
*
*
*
*
1
5
10
25
50
100
Fenoldopam Dosage (µg/kg/min)

Figure 8

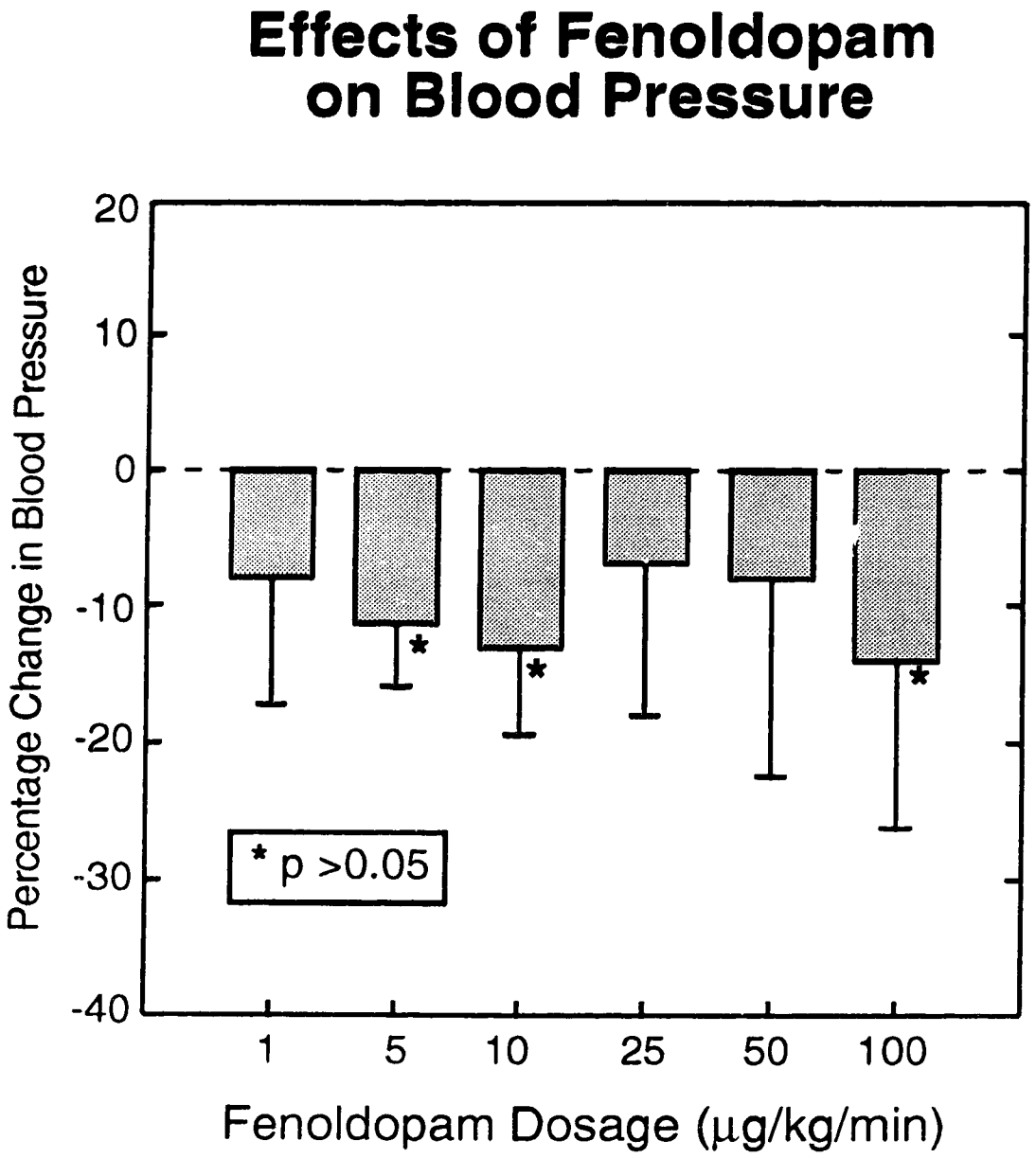

Effects of Fenoldopam on Blood Pressure
Percentage Change in Blood Pressure
20
10
0
-10
-20
-30
-40
* p >0.05
1
5
10
25
50
100
Fenoldopam Dosage (µg/kg/min)

Figure 9

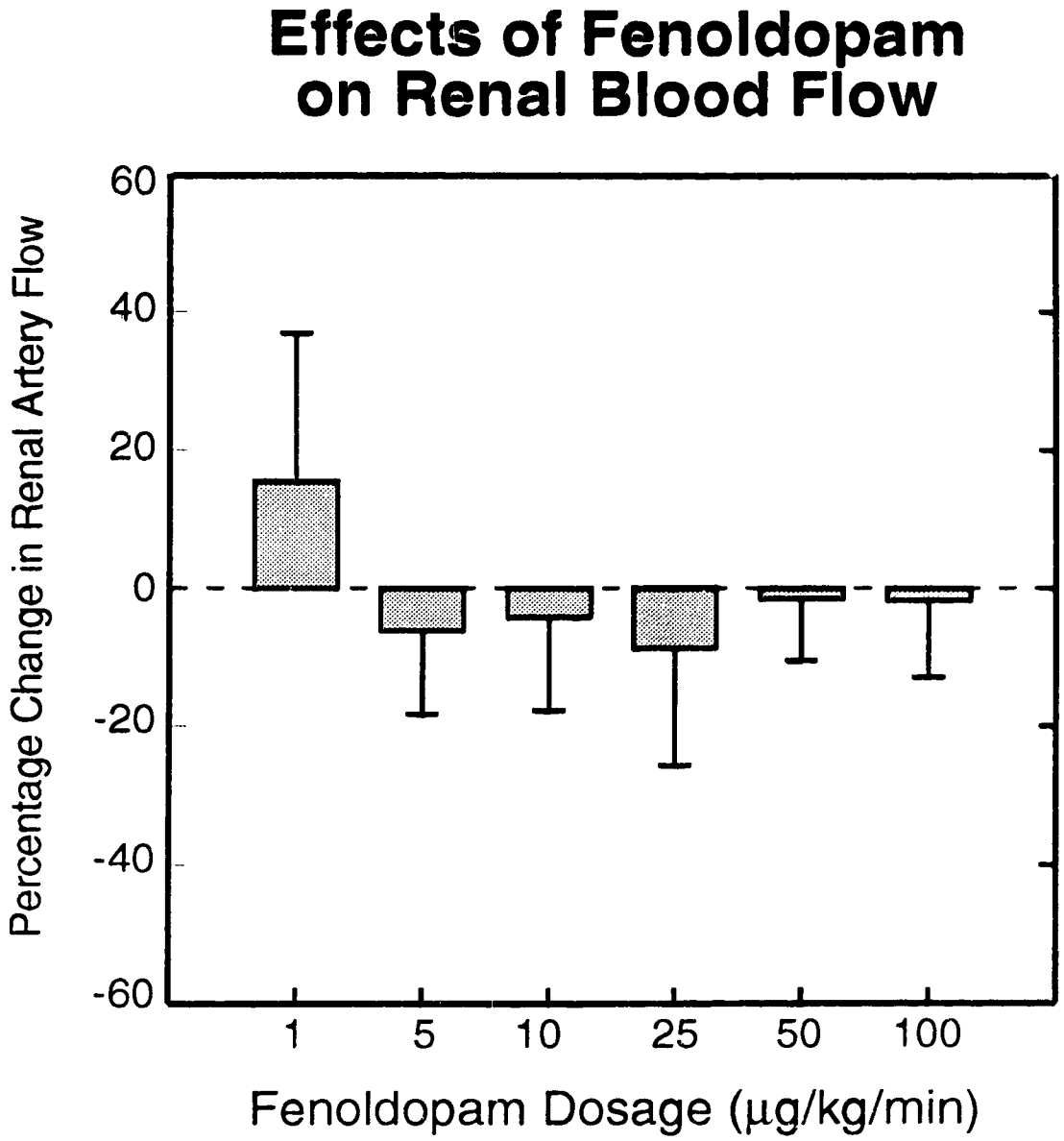
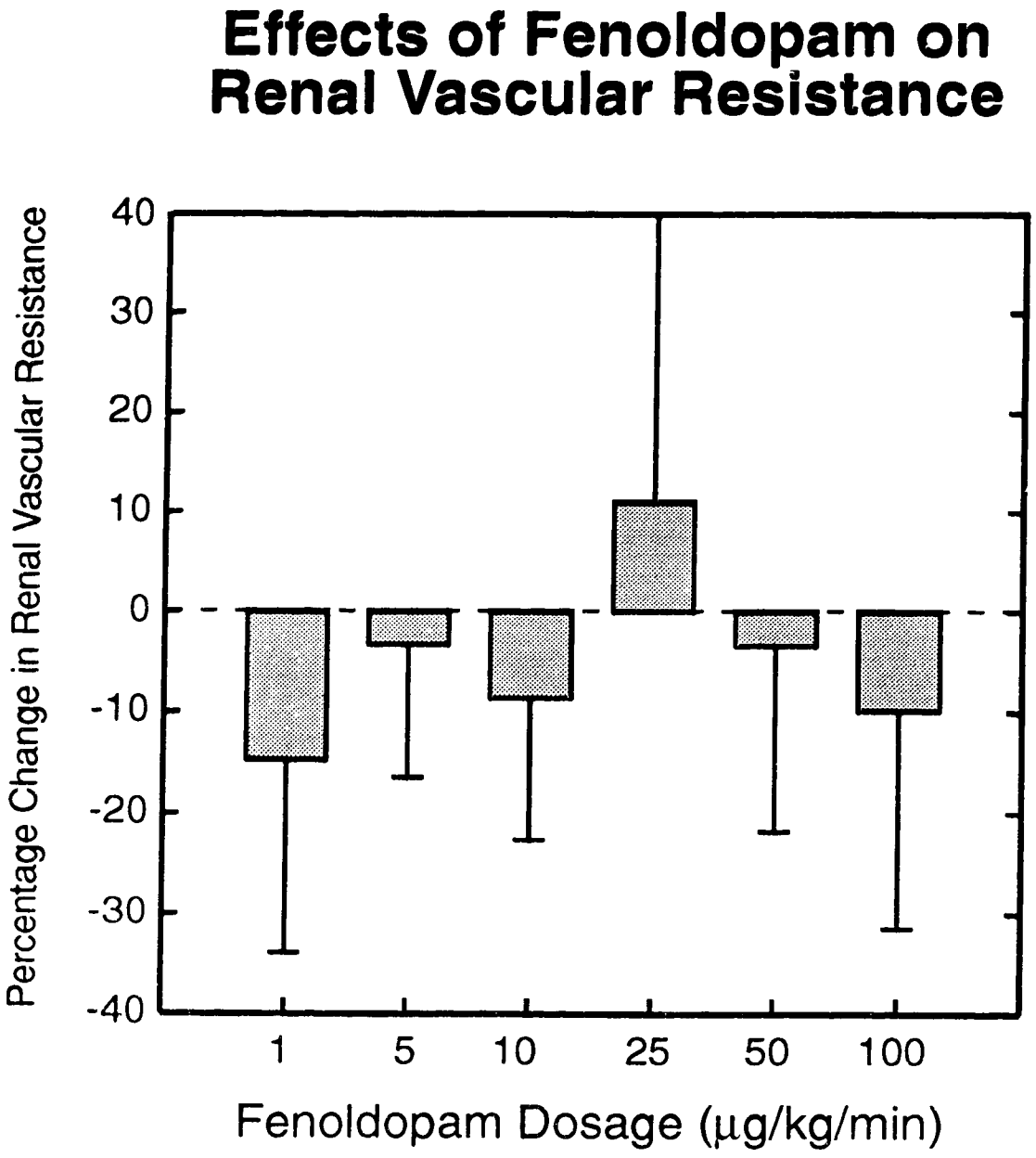
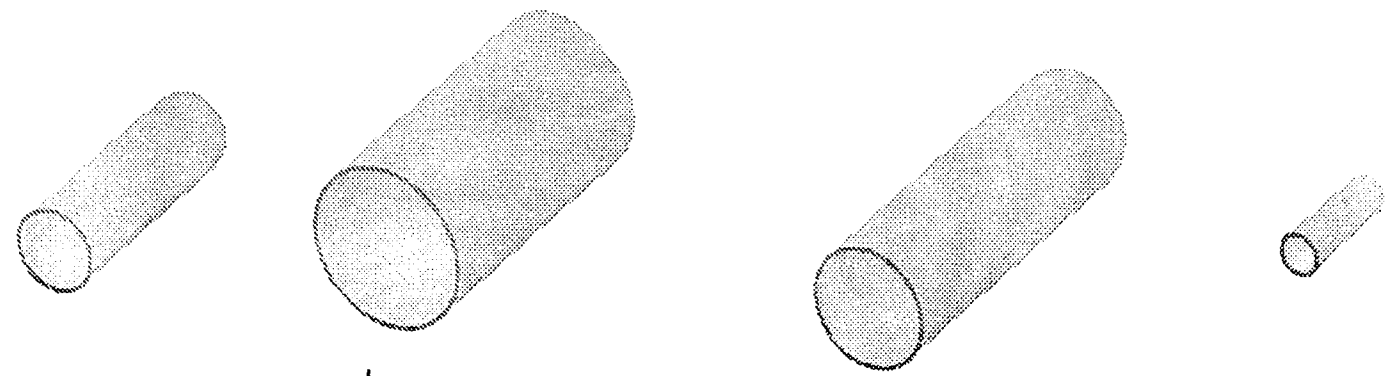

Effects of Fenoldopam on Renal Blood Flow
Percentage Change in Renal Artery Flow
60
40
20
0
-20
-40
-60
1
5
10
25
50
100
Fenoldopam Dosage (µg/kg/min)

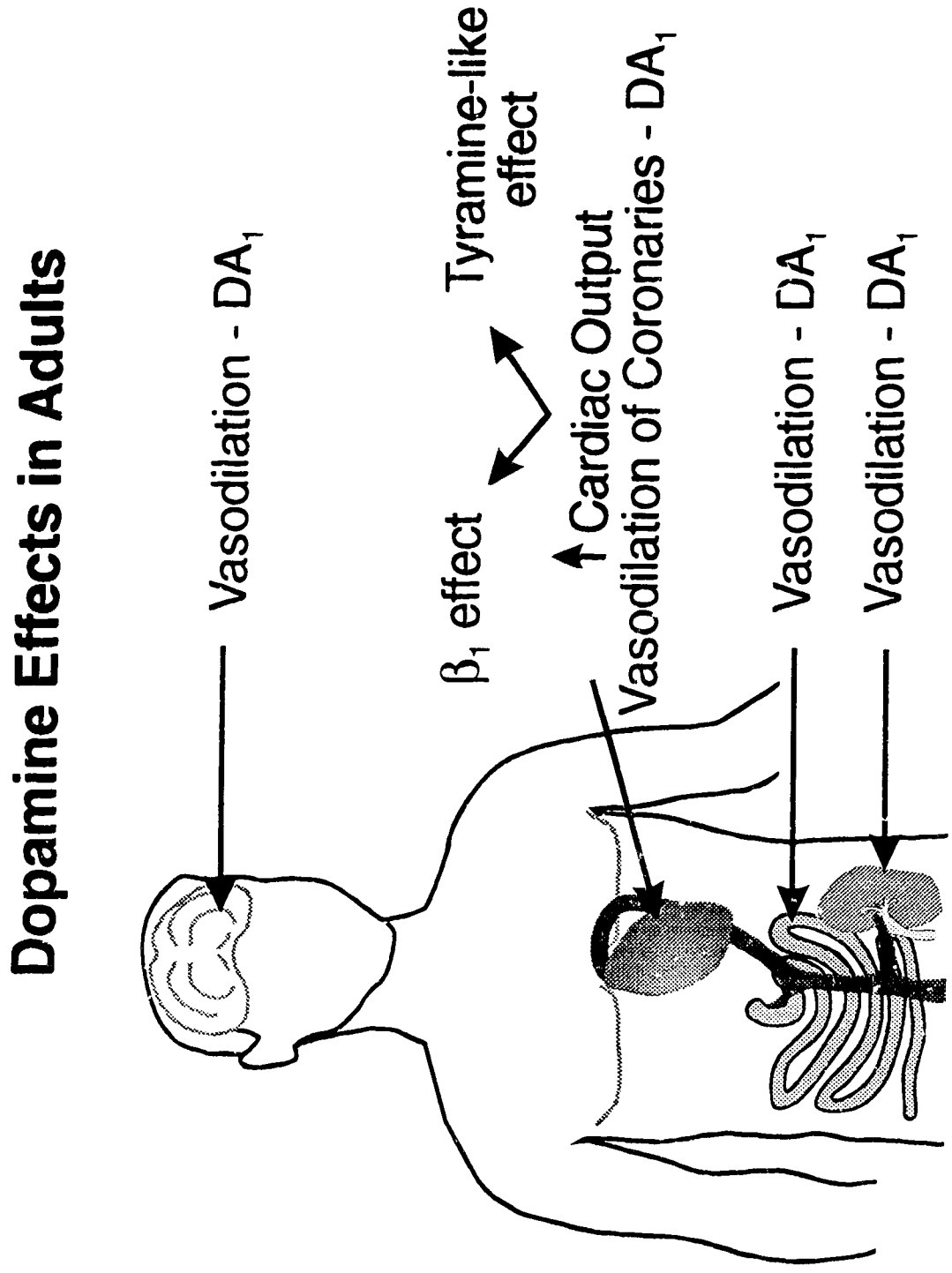
Figure 10


Effects of Fenoldopam on Renal Vascular Resistance
Percentage Change in Renal Vascular Resistance
40
30
20
10
0
-10
-20
-30
-40
1
5
10
25
50
100
Fenoldopam Dosage (µg/kg/min)

Vascular Response to Dopamine in Adults

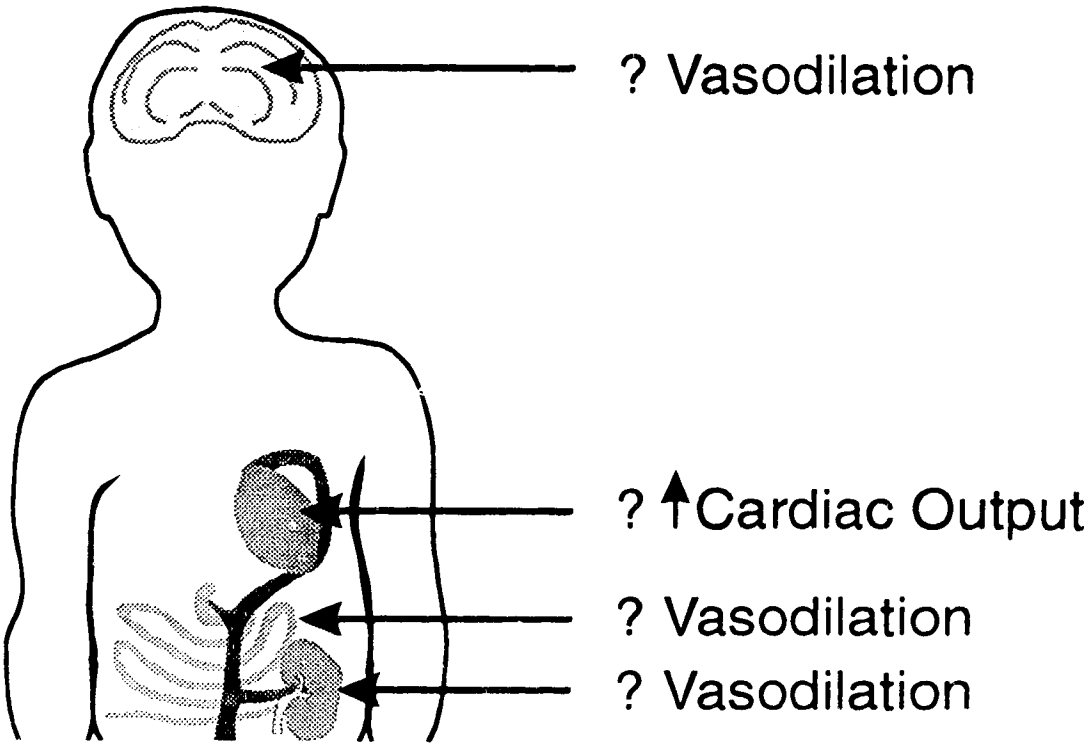


Appendix 2

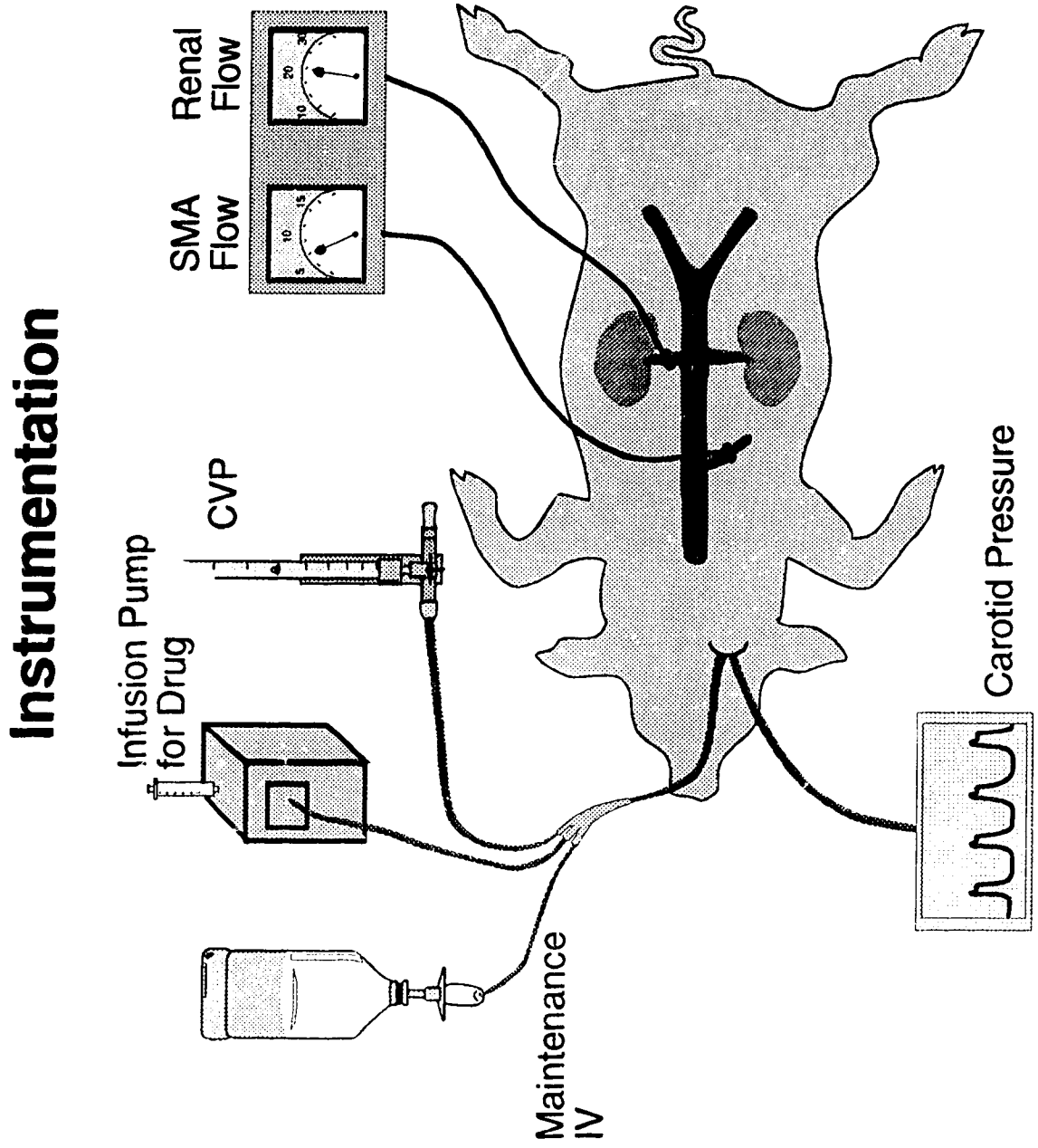

Dopamine Effects in Adults
Vasodilation - DA1
Tyramine-like effect
β1 effect
Cardiac Output
Vasodilation of Coronaries - DA1
Vasodilation - DA1
Vasodilation - DA1

Appendix 3

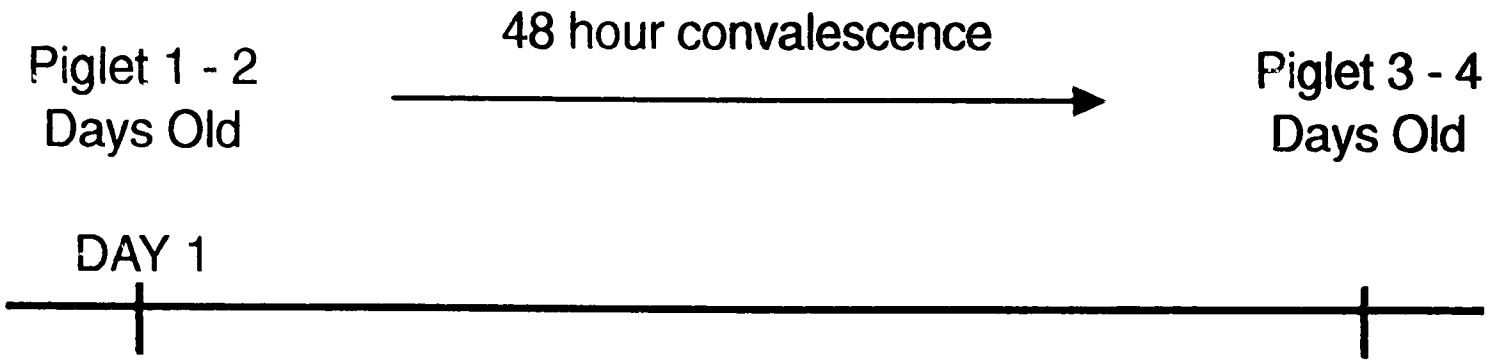
Dopamine Effects in Newborns



Appendix 4


Instrumentation
Renal Flow
SMA Flow
CVP
Infusion Pump for Drug
Carotid Pressure
Maintenance IV

Experimental Methods I



Instrumentation:

- Venous double or triple lumen catheter
- Arterial line
- 2 x ultrasonic flow probes -
  for renal and sup. mesenteric
  arteries.

Experiment:

- Conscious piglet
- Dose / response
  recording with either:
  - ➤ Dopamine (2-32 µg/kg/min)
  - ➤ Fenoldopam (1-100 µg/kg/min)

Appendix 6

Experimental Methods II