1984

The Effect of Benzodiazepines on the Stress Response to Drug-Induced Hypotension and Surgical Trauma

Gail Elizabeth Gillenwater
Loyola University Chicago

THE EFFECT OF BENZODIAZEPINES ON THE STRESS RESPONSE TO DRUG-INDUCED HYPOTENSION AND SURGICAL TRAUMA

by

GAIL ELIZABETH GILLENWATER

A Dissertation Submitted to the Faculty of the Graduate School of Loyola University of Chicago in Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

JANUARY

1984

ACKNOWLEDGEMENTS

I would like to thank Dr. Silas Glisson, chairman of my dissertation committee, for his generous assistance and helpful advice in all aspects of the Ph.D. pathway, and most especially in the design of the experiments, development of laboratory techniques and preparation of the manuscript.

I would like to thank each member of my dissertation committee: Drs. Alexander Karczmar, Adel El-Etr, John Zaroslinski and Stephen Jones, for their valuable comments and suggestions, without which this dissertation would have been a lesser work.

I would like to thank Maggie Kubak and Mark Hieber, who were generous with both technical assistance and friendship.

I would like to thank Jack Corliss and other members of Academic Computing Services for their assistance with the statistics, graphics and word processing of this dissertation.

I would like to thank Jan Mixter and other members of the library staff for their kind helpfulness in the many services that they provide to the graduate student.

Finally, I would like to thank Drs. Paul Ellen, Kirk Richardson and Duane Rumbaugh of Georgia State University who, as my first graduate educators, instilled in me a love for the truth and beauty of Science.

To Follow Knowledge Like a Sinking Star,

Beyond the Utmost Bound of Human Thought.

Alfred Lord Tennyson

"Ulysses"

VITA

Gail Elizabeth Gillenwater was born in Logan, West Virginia in 1950. She attended primary school in Martinsburg, West Virginia and graduated from Dulaney Valley High School in Timmonium, Maryland in 1968.

After graduation from high school, Gail attended Wake Forest University, where she was awarded a scholarship to study at the University of the Andes in Bogota, Colombia. On returning to the United States, Gail attended Michigan State University. At Michigan State she was admitted to the Honors College, joined Phi Beta Kappa and Phi Kappa Phi, and in 1972 she was awarded a Bachelor of Arts degree with High Honor.

Gail began postgraduate work in 1973 in the Department of Psychology at Georgia State University. At Georgia State, Gail's research on animals with septal lesions was conducted in the laboratory of Dr. Paul Ellen. She received a Master of Arts degree from Georgia State in 1976.

In 1979 Gail was admitted to the Department of Pharmacology at Loyola University where she was granted a Basic Science Fellowship. Her research was conducted in the labo-

ratory of Dr. Silas Glisson, and in 1984 she was granted the degree of Doctor of Philosophy.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CONTENTS OF APPENDICES

LIST OF ABBREVIATIONS

ACTH	adrenocorticotropic hormone
ANOVA	analysis of variance
BSA	body surface area
CI	cardiac index
cm	centimeter
CO	cardiac output
CRF	corticotropin releasing factor
C.V.	coefficient of variation
DAP	diastolic arterial pressure
dl	deciliter
drug*time	drug times time
ECG	electrocardiogram
EDTA	ethylenedinitrilo-tetraacetic acid
GABA	gamma-aminobutyric acid
gm	gram
GMP	guanosine monophosphate
Hg	mercury
HR	heart rate
hr	hour
Hz	hertz
I	iodine
i.v.	intravenous
kg	kilogram
L	liter
LVSWI	left ventricular stroke work index
M	meter
MAC	minimum alveolar concentration
MAP	mean arterial pressure
mcg	microgram
mcl	microliter
mg	milligram
min	minute
ml	milliliter
mm	millimeter
MSBG	mean square between group
MSWG	mean square within group
N	normal
ng	nanogram
nM	nanometer
p	probability
PADP	pulmonary artery diastolic pressure
PASP	pulmonary artery systolic pressure

PCA	perchloric acid
PCWP	pulmonary capillary wedge pressure
RAP	right atrial pressure
RIA	radioimmunoassay
RPM	revolutions per minute
SAP	systolic arterial pressure
sec	second
SI	stroke index
SVRI	systemic vascular resistance index

CHAPTER I

INTRODUCTION

Perioperative Stress and Physiological Responses

Patients undergoing cardiac surgery or major noncardiac surgery are exposed to a variety of preoperative, intraoperative and postoperative stresses. During general surgical procedures, the patient is stressed by presurgical anxiety, induction of anesthesia, intubation, surgical trauma and a variety of medications administered during the course of the operation. The cardiac surgery patient, while being subjected to the usual surgical stresses, is further stressed during the cardiopulmonary bypass period when his cardiorespiratory functions are taken over by the heart-lung bypass machine and abrupt physiological changes such as hypotension, hypothermia and hemodilution occur. The response of the body to stress induced by surgical trauma, antihypertensive drugs or extracorporeal circulation involves characteristic changes in plasma catecholamines, cortisol and renin activity.

Surgical Stress

The effect of surgical stress on plasma catecholamines has been actively debated in recent years. Hammond et al. (1956) found no systematic alterations of plasma catecholamines in patients undergoing uneventful major surgery. Hine et al. (1976) reported no significant elevations in plasma catecholamines following induction of anesthesia, skin incision or thoracotomy in cardiac surgery patients; however, elevations in both epinephrine and norepinephrine were seen late into the cardiopulmonary bypass period. Butler et al. (1977), like the Hine group, found no significant increases in plasma catecholamines during the early phases of cardiac surgery, but elevations in both epinephrine and norepinephrine were seen during the bypass period. This group failed to find any significant changes in plasma catecholamines following abdominal surgery.

Many studies have shown that plasma catecholamines are elevated in response to intraoperative stress. Anton et al. (1964) reported elevated catecholamines in patients during both thoracotomy and cardiopulmonary bypass procedures. There were also several reports of elevated catecholamines

during the cardiopulmonary bypass period in patients undergoing myocardial revascularization (Balasaraswathi et al., 1978 and 1980; Tan et al., 1978; Hoar et al., 1980a; Kim et al. 1981).

A variety of plasma catecholamine responses has been reported in patients undergoing abdominal surgery and other lower body procedures. Elevated plasma epinephrine and norepinephrine levels (Halter et al., 1977), elevated epinephrine levels (Madsen et al., 1978) and elevated norepinephrine levels (Pflug and Halter, 1981) have been shown.

Thus, while some reports conclude that surgical stress does not consistently elevate plasma catecholamines, a substantial body of evidence indicates that elevations in plasma catecholamines occur in response to surgical stress.

Several investigators have attempted to clarify the effects of surgical stress on plasma catecholamines. Balasaraswathi et al. (1978) found changes in both epinephrine and norepinephrine paralleled changes in blood pressure during coronary artery bypass surgery. These authors suggested that the elevated plasma catecholamine levels were most likely due to a sympathetic response to hypotension. Kim et al. (1981) also found elevated norepinephrine and epinephrine levels during cardiopulmonary bypass; however, they did not find as marked an increase in epinephrine as norepineph-

rine. The authors suggested that changes in norepinephrine rather than epinephrine are responsible for the hemodynamic changes occurring during cardiopulmonary bypass. But, in a direct contradiction of Kim's results, Reves et al. (1982) found that it was epinephrine rather than norepinephrine that showed the greatest elevation in response to cardiopulmonary bypass, and these authors suggested that epinephrine release is the predominant humoral response to cardiopulmonary bypass surgery.

Both norepinephrine and epinephrine have been implicated in hemodynamic changes occurring during surgery; however, the precise role of each has not been clearly defined.

Anesthesia

Anesthetics do not have a singular effect on plasma catecholamine levels. Most anesthetics either depress or do not affect plasma catecholamines when measured shortly after induction in patients undergoing surgical procedures. Halothane depressed epinephrine and norepinephrine levels in several studies (Perry et al., 1974; Roizen et al., 1974; Balasaraswathi et al. 1980). Enflurane either depressed (Kim et al., 1981) or did not affect plasma epinephrine and norepinephrine levels (Balasaraswathi et al., 1978; Tan et al., 1978). On the other hand, morphine either elevated

(Hasbrouck, 1970) or did not affect plasma catecholamine levels (Balasaraswathi et al., 1978; Tan et al., 1978). Isoflurane has been reported to depress both epinephrine and norepinephrine (Perry et al., 1974) or to elevate epinephrine and depress norepinephrine levels (Balasaraswathi et al., 1982). Zsigmond (1974) reported an increase in plasma norepinephrine levels following ketamine.

Clinical Application

Elevated plasma catecholamines, as well as elevated cortisol and renin activity, have been associated with postoperative hypertension, which may present a serious problem to cardiac surgery patients. Hypertension in the immediate postoperative period following myocardial revascularization has been reported to occur in 33% (Estafanous et al., 1973) to 58% (Hoar et al., 1976) of these patients. Untreated and sustained hypertension can have deleterious effects in certain patients. Some of these dangers include bleeding from deep and superficial incisions, leakage at suture lines, and an increase in the susceptibility of patients with generalized atherosclerosis to cerebrovascular accidents (Viljoen et al., 1976).

Van Ackern et al. (1979) advised that patients with coronary artery disease should be protected against hyper-

tensive episodes; the risk of myocardial infarction is increased when an oxygen imbalance exists in the coronary artery supply-demand relationship. Hypertension, which increases the oxygen demand by increasing the work load of the heart, adversely affects myocardial oxygenation and is potentially harmful to patients with ischemic heart disease. In the same vein, Hoar et al. (1980a) have suggested that the fall in blood pressure that often follows anesthesia induction may be beneficial to patients with ischemic heart disease.

Hypertension can have adverse effects on patients undergoing surgical correction of cerebral aneurysms as well as on patients with impaired heart function. That is, the rupture of an intracranial aneurysm can result in immediate or subsequent fatal hemorrhage (Skultety and Nishioka, 1966). Because nitroprusside is frequently used to induce hypotension for correction of intracranial aneurysms, rebound hypertension occurring on discontinuation of nitroprusside can jeopardize the patient's recovery (Skultety and Nishioka, 1966).

In cardiac surgery, elevated catecholamine levels may reflect a reflex response to the hypotension induced during the initial cardiopulmonary bypass period. This reflex response is thought to be mediated via the baroreceptors,

since they play a key role in short-term adjustments of blood pressure. Baroreceptors, which are stretch receptors located in the carotid sinus and aortic arch, are sensitive to changes in blood pressure (Berne and Levy, 1977). A reduction in blood pressure results in a reduction in the baroreceptor firing rate. This activates the medullary vasomotor center, increasing sympathetic activity and catecholamine release, which then leads to an increase in blood pressure and heart rate.

Elevated plasma catecholamine levels during cardiac surgery might also be associated with the period of hypothermia to which the patient is exposed during surgery. Hypothermia, which slows the heart and depresses the body's metabolism, causes the hypothalamic temperature-regulating center to be activated. This leads to increased sympathetic activity, resulting in vasoconstriction and heat conservation (Guyton, 1976). Plasma catecholamine levels are increased during normothermic (33-37°C) and moderate hypothermic (27-31°C) cardiopulmonary bypass, but less so with the latter. With deep hypothermia (<27°C) the epinephrine increase does not occur. Plasma norepinephrine levels increase similiar to moderate hypothermia (Replogle et al., 1962, Anton et al., 1964).

During noncardiac abdominal surgery Halter et al. (1977) reported that changes in epinephrine and norepinephrine correlated well with changes in mean arterial pressure. These authors suggested that during this type of surgery, hypertension probably reflects an adrenergic response to pain rather than a reflex reponse to hypotension. In any event, in both cardiac and noncardiac surgery, elevations in plasma catecholamines have been associated with hypertensive episodes.

Elevated catecholamine levels, which may be harmful to certain patients, may be functionally necessary for others. Increased sympathetic activity increases both heart rate and the contractile strength of the heart. Patients who require increased sympathetic activity for sufficient cardiac output, i.e. valvular disease, could experience reduced cardiac performance if catecholamine levels were lowered.

In addition to the effects of catecholamines on blood pressure, several investigators have attempted to correlate plasma catecholamines with other physiological conditions. The results of the Vlachakis et al. (1981) study suggests that elevated plasma catecholamines may increase platelet aggregation and consequently promote postoperative thromboembolism. Other investigators, however, have not been successful in correlating plasma catecholamine levels with

physiological conditions. Halter et al. (1977) was unable to correlate changes in epinephrine and norepinephrine during abdominal surgery to hypoxemia or hypothermia. Furthermore, Turton et al. (1977) found no correlation between elevated plasma catecholamine levels and mean diastolic pressure, heart rate or respiration rate.

In conclusion, changes in plasma catecholamines may reflect the response of the body to many surgical and nonsurgical stresses. The exact role that elevated catecholamines play in homeostasis following intraoperative and postoperative stresses has not been clearly defined. However, elevations in plasma catecholamines can be either harmful or beneficial, depending on the interaction between the cardiac status of the patient and type of surgical stress that he is scheduled to undergo.

Cortisol

Surgical Stress

Cortisol has consistently been shown to be elevated in response to surgical stress. With regard to noncardiac surgery, elevated plasma cortisol has been reported during

abdominal surgery (Lewis, 1963; Plumpton and Besser, 1969; Bromage et al., 1971; Newsome and Rose, 1971; Cosgrove and Jenkins, 1974; Kehlet et al., 1974; Madsen et al., 1976 and 1977; Engquist et al., 1977), thoracic surgery (Bromage et al., 1971), hip surgery (Plumpton and Besser, 1969), tympanoplasty (Madsen et al., 1976) and in the undefined category of general surgery (Reier et al., 1973; Gill et al., 1975; Oyama et al., 1975). Plumpton and Besser (1969) studied the long-term response of cortisol to noncardiac surgery by monitoring plasma cortisol levels hourly during major surgery and then daily for six days postoperatively. These authors found a rapid rise in plasma cortisol levels during surgery, a continued rise during the first six hours of the postoperative period, and then a gradual decrease over the next 24-48 hours.

Madsen et al. (1976) reported that the magnitude of elevated plasma cortisol levels is related to the intensity of the surgical stress. These authors measured plasma cortisol levels during two different types of surgery and found that plasma cortisol levels during hysterectomy were significantly higher than cortisol levels during tympanoplasty.

The cortisol response during cardiac surgery was demonstrated by Taylor et al. (1976). These authors compared plasma cortisol levels in patients undergoing cardiac sur-

gery either with or without the use of heart-lung bypass. In the nonbypass patients, the cortisol response to cardiac surgery was similar to that previously described for noncardiac surgery, with the levels peaking late into the operative procedure and then gradually decreasing over the next 48 hours. In bypass patients, plasma cortisol levels were elevated following surgical stress, decreased during the bypass period, peaked 24 hours after the operation, and remained elevated 48 hours postoperatively.

Oka et al. (1981) compared plasma cortisol levels in patients undergoing cardiac surgery for valvular or coronary artery bypass surgery. In accordance with the results of the Taylor et al. (1976) study, coronary artery bypass patients showed increased plasma cortisol levels in response to surgery and these levels continued to rise postoperatively. The response of the valvular surgery patients, however, differed from that of the coronary artery bypass patients in both absolute levels and pattern of response. That is, valvular surgery patients showed lower plasma cortisol levels than coronary artery bypass patients. Also the peak cortisol response in valvular surgery patients was seen at the end of the cardiopulmonary bypass period rather than late into the postoperative period.

Plasma cortisol levels do increase in patients undergoing both cardiac and noncardiac surgery. Furthermore, the pattern of the cortisol response varies with different surgical procedures. Also, steriods have been demonstrated to interfer with nonneuronal catecholamine reuptake (Iverson and Salt, 1970), suggesting that plasma catecholamine levels may be influenced by cortisol. Furthermore, recent evidence suggests that in the periphery uptake 2 may have a more important role than uptake 1 (Chan and Kalsner, 1982).

Anesthesia

The effects of general anesthesia on plasma cortisol levels for both cardiac and noncardiac surgery patients have been reported by many investigators. These studies have shown that cortisol levels remained relatively stable following the administration of a variety of anesthetic agents including enflurane, nitrous oxide-oxygen-d-tubocurare, halothane, isoflurane and nitrous oxide-oxygen-fentanyl (Lewis, 1963; Oyama et al., 1975; Yokota et al., 1977; Oyama et al., 1979; Oka et al., 1981). The poststress elevations in plasma cortisol levels are probably related to the stress itself, rather than to the anesthetic agent administered to the patient.

Clinical Application

Oka et al. (1981) have suggested that plasma cortisol levels following surgery sometimes may be a factor in the patients' ultimate prognosis. They suggest that the relatively poor postoperative recovery seen in critically ill valvular surgery patients as compared to that of patients undergoing coronary artery bypass surgery may be related to lower plasma cortisol levels in valvular surgery patients. Also, it is well known that patients with adrenocortical insufficiency often experience cardiovascular instability during surgery without prior administration of glucocorticoids.

On the other hand, elevated plasma cortisol levels may not always be beneficial to patients undergoing the stress of surgery. In patients with hyperadrenocortical function elevated cortisol levels may be harmful. Madsen et al. (1977) have suggested that patients suffering from labile diabetes have a high surgical morbidity and might benefit from a reduction in cortisol levels. Furthermore, Turndorf (1973) has suggested that very high cortisol levels might be "antihomeostatic" since the highest cortisol levels in the Reier et al. study (1973) occurred in "nonsurvivors."

Thus, plasma cortisol levels have been shown to increase in response to both cardiac and noncardiac surgery.

Although increased cortisol levels may be beneficial to certain surgical patients, there are instances where such elevations may actually be detrimental to the patients' recovery.

Renin Activity

The renin-angiotensin system plays an important role in maintaining arterial blood pressure, fluid volume and electrolyte balance. Although renin itself is not a vasoactive substance, it acts in the general circulation to generate angiotensin I, which is in turn converted to angiotensin II, a very potent vasoconstrictor. Because an inexpensive and reliable assay for angiotensin II has not yet been developed, plasma renin activity rather than plasma angiotensin II levels are frequently taken to reflect renin-angiotensin system activity.

Surgical Stress

Both cardiac and noncardiac surgery have been associated with increased plasma renin activity (Yogo et al., 1973; Favre et al., 1974; Oyama et al., 1979; Philbin et

al., 1979; Lappas et al., 1981). In both cardiac and abdominal surgery, the response of the renin-angiotensin system to surgical stress tends to be somewhat delayed. Oyama et al. (1979) found that plasma renin activity was not increased until 30 minutes after abdominal surgery. A similar delay was reported by Philbin et al. (1979) in patients undergoing cardiac surgery. Also Favre et al. (1974) reported a marked increase in plasma renin activity, but not until 20 minutes after the onset of cardiopulmonary bypass.

Not all investigators, however, have reported elevated plasma renin activity following surgical stimulation. Hoar et al. (1980a) did not find significant elevations in plasma renin activity either following surgical stimulation or during cardiopulmonary bypass. Their patients, however, had been pretreated with propranolol, which has been shown to block renin release (Buhler, 1980). Moreover, Yun et al. (1979) did not observe a rise in plasma renin activity after laparotomy, but the anesthetic used in this study was pentobarbital, which the authors point out decreases plasma renin activity over an extended period of time (Yun et al., 1979).

Anesthesia

Many anesthetic agents do not significantly affect plasma renin activity. For example, enflurane, methoxyflurane, ether and morphine neither increase nor decrease plasma renin activity (Bailey et al., 1975; Miller et al., 1977; Oyama et al., 1979). Elevations in plasma renin activity, however, were reported in one study following halothane anesthesia (Oyama et al., 1979). Yun et al. (1979) reported that pentobarbital had a biphasic effect on plasma renin activity, increasing during the first hour after injection and decreasing for the next 4-1/2 hours.

Clinical Application

The results of several studies suggest that elevated plasma renin activity might be involved in perioperative hypertension. Taylor et al. (1977) compared plasma angiotensin II levels in patients undergoing cardiac surgery with and without cardiopulmonary bypass. These authors found a marked rise in plasma angiotensin II levels in the bypass patients that persisted over two hours postoperatively. Because hypertension is often seen in the first few hours after cardiac bypass surgery, they proposed that elevated angiotensin II levels produce the vasopressor response and subsequent hypertension.

Roberts et al. (1977) also presented evidence to suggest that increased renin-angiotensin system activity might be a factor in systemic hypertension seen during coronary artery bypass surgery. In this study, patients who became hypertensive during surgery had higher plasma renin activity levels than patients who did not develop hypertension. Also, these authors found a positive correlation between mean arterial pressure and plasma renin activity. The evidence suggests that the renin-angiotensin system may play a role in perioperative hypertension which can pose a risk to certain surgical patients.

In addition to the elevated plasma renin activity seen following the stress of surgery and cardiopulmonary bypass, plasma renin activity often increases following nitroprusside-induced hypotension (Kaneko et al., 1967; Miller et al., 1977; Abukhres et al., 1979; Khambatta et al., 1979; Cottrell et al., 1980; Delaney and Miller, 1980; Stanek et al., 1981). In some patients, following termination of the nitroprusside infusion, rebound hypertension occurs. Plasma renin activity may be an important factor mediating this rebound hypertension. Cottrell et al., (1980) state that the half-life of renin activity is approximatley 30 minutes and the half-life of nitroprusside is only 1-2 minutes. The authors suggest that the rebound hypertension

following nitroprusside-induced hypotension probably results from the slow removal of renin from the circulation as compared with the rapid clearance of nitroprusside. Contraction of vascular smooth muscle then no longer would be countered by nitroprusside-induced vasodilation.

Further support for the involvement of the renin-angiotensin system in this rebound hypertension comes from Abukhres et al. (1979). These authors showed that rebound hypertension did not occur when the primary source of renin was eliminated by nephrectomy. They also showed that the rebound hypertension was markedly attenuated when renin release was blocked by propranolol or when generation of angiotensin II was blocked with a converting-enzyme inhibitor.

Other investigators have supported the position that renin release rather than sympathetic stimulation causes the rebound hypertension following a nitroprusside infusion. Cottrell et al. (1980) concluded that catecholamines are probably not involved in this rebound hypertension because tachycardia was not associated with the elevated blood pressure. Moreover, Rawlinson et al., (1978) showed that although epineprhine and norepinephrine were elevated during nitroprusside infusion, both approached preinfusion levels 15 minutes after the infusion ended. The Rawlinson evidence

is not conclusive, however, because these authors did not report mean arterial pressure following the termination of the nitroprusside infusion. Rebound hypertension may not have occurred in their patients. In a later study, Delaney and Miller (1980) found that while nitroprusside was being infused at a constant rate, blood pressure fell significantly and then progressively increased. However, when the renin-angiotensin system was blocked with saralasin, an angiotensin II antagonist, blood pressure remained at a constant depressed level during the nitroprusside infusion and no rebound hypertension was seen. Because no increase in blood pressure was seen during renin-angiotensin system blockade, these authors concluded that "other means of blood pressure support (e.g., catecholamines) are not operative in this setting" (p. 155). However, catecholamines were not measured in this study. Angiotensin II is known to facilitate sympathetic transmission (Swales, 1979); thus, blockade of angiotensin II could have also attenuated the catecholamine response to nitroprusside.

In summary, plasma renin activity has been shown to be elevated in response to surgical and hypotensive stresses, but not in response to most anesthetics. Also, the renin-angiotensin system probably plays an important role in the hypertension that often occurs following surgical stimula-

tion and nitroprusside infusions. Because hypertensive episodes can be harmful in certain clinical settings, it may be beneficial to pharmacologically blunt such untoward rises in plasma renin activity.

Attenuation of Stress Responses by Benzodiazepines

The ability to manipulate plasma catecholamine, cortisol and renin activity levels may be of benefit in the clinical management of some patients. In light of the previously discussed theoretical association between elevated blood pressure and elevated plasma catecholamines and renin activity, surgical patients with ischemic heart disease might benefit from an attenuation of stress-induced elevations in catecholamines (Van Ackern et al., 1979; Hoar et al., 1980a) or renin activity (Taylor et al., 1977; Roberts et al., 1977). Also, elevated blood pressure can be especially harmful to patients with intracranial aneurysms (Skultety and Nishioka, 1966), and in the perioperative period these patients might benefit from pharmacologically blunting elevated plasma catecholamines and renin activity. Moreover, surgical patients with hyperadrenocortical function or labile diabetics might benefit from a reduction in stress-induced elevations in plasma cortisol levels (Madsen et al., 1977). The results of several studies suggest that the benzodiazepines might effectively antagonize these compensatory hormonal responses which often occur during the course of surgery.

Pharmacological Profile of Diazepam, Lorazepam and Midazolam

Diazepam

Chemistry and Pharmacology

Diazepam, which is probably the most widely used benzodiazepine, has a variety of therapeutic actions. Diazepam has been used for producing mild sedation and managing anxiety, tension and convulsive states, and more recently it has been used as a premedicant and as an adjuvant to intravenous anesthesia.

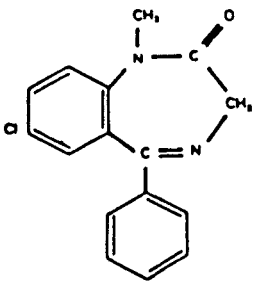
Pharmacokinetic studies indicate that diazepam has a rapid onset of action and a prolonged duration of action. Jones et al. (1979) reported induction of anesthesia within 94-158 seconds of diazepam injections, and Hillestad et al. (1974) found that within 15 minutes of diazepam injections, maximum clinical effects reflected in sleepiness, coordination difficulties, mental clouding and amnesia were recorded. The prolonged effects of diazepam are probably due to an elimination half-life of 21 to 37 hours, a very large volume of distribution (0.95 L/kg) and slow clearance rate (0.35 ml/min/kg) as well as extensive (96.8%) plasma

protein binding (Kaplan et al., 1973; Klotz et al., 1976). Furthermore, certain tissues of the brain, kidney, liver, myocardium and digestive system have been shown to rapidly take up diazepam and then slowly release it (Mandelli et al., 1978).

The biotransformation of diazepam leads to the formation of two important metabolites, desmethyldiazepam and oxazepam, both of which are active (Kaplan et al., 1973; Mandelli et al., 1978). Desmethyldiazepam, the major metabolite measured in the bloodstream, is formed by demethylation of diazepam and has an elimination half-life of 50-99 hours, which is even longer than that of diazepam. Oxazepam, formed primarily from hydroxylation of desmethyldiazepam, undergoes glucuronide conjugation and this then becomes the major urinary metabolite.

A distinct disadvantage of diazepam is that it is not water soluble. Consequently, injectable diazepam is prepared in an organic solvent such as propylene glycol.

The strucutral formula of diazepam is:



Cardiovascular and Respiratory Effects

Numerous studies have indicated that the circulatory and respiratory effects of diazepam are mild and often clinically insignificant. In an early study, no significant decreases in respiration rate, heart rate, systolic and diastolic blood pressures, or changes in arterial blood chemistry were found in patients premedicated and induced to anesthesia with diazepam (McClish, 1966). The only complications noted in this study were several complaints of pain on injection and a small percentage of cases of venous thrombosis and thrombophlebitis at the site of injection. Later studies tended to confirm the lack of deleterious effects of diazepam on the cardiovascular and respiratory systems. When statistically significant decreases in heart

or lung function were found, as reflected most often in decreases in blood pressure or heart rate, these effects were not considered to be clinically significant (Cote et al., 1974; Markiewicz et al., 1976; McCammon et al., 1980; Samuelson et al., 1981).

The results of several studies have suggested that diazepam might actually be beneficial to some cardiac surgery patients. Ikram et al. (1973) measured myocardial blood flow by the ^{133}Xe clearance method and showed that diazepam increased myocardial blood flow in patients with diseased and normal coronary arteries. On the other hand, Cote et al. (1974) measured myocardial blood flow by thermodilution in a similiar patient population, but they did not find that diazepam increased coronary blood flow. The inconsistancy in the results of the Ikram and Cote studies is probably a function of the different methods used in these two studies to measure coronary blood flow.

A decrease in myocardial oxygen consumption was reported by Cote et al. (1974). Daniell (1975) confirmed these results, and also found that diazepam treatment was associated with an increase in coronary blood flow.

In summary, diazepam appears to be devoid of clinically significant untoward hemodynamic or respiratory effects. Furthermore, the reported increase in myocardial

blood flow and decrease in oxygen consumption suggest that diazepam would be a good premedicant for certain patients with compromised heart or lung function.

Lorazepam

Chemistry and Pharmacology

Lorazepam is a benzodiazepine that resembles diazepam in many of its clinical effects. Like diazepam, lorazepam has sedative-hypnotic, muscle relaxant, antianxiety and anticonvulsant properties, and is currently being used as a premedicant for general anesthesia (Alps et al., 1973; Wilson, 1973; Conner et al., 1978; Ameer and Greenblatt, 1981). The clinical effects of lorazepam are quite similar to those of diazepam. Lorazepam is four to five times more potent than diazepam (Dundee et al., 1979), only 88-92% bound to plasma protein (Greenblatt, 1981), and there are distinct pharmacological differences between these two benzodiazepines.

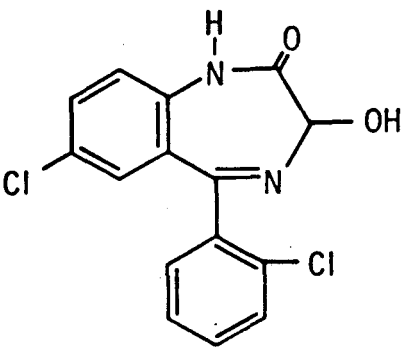
Lorazepam has a considerably longer onset and duration of action than diazepam. Conner et al. (1978) compared the onset of the sedative and antianxiety effects of equipotent doses of lorazepam and diazepam and found that the diazepam

group showed a peak effect in four minutes, but the lorazepam peak was not seen for 32 minutes. Conner et al. concluded that while the peak effects of diazepam and lorazepam did not differ significantly, the time taken to achieve the peak effect was significantly delayed in the lorazepam group. These results were confirmed by Dundee et al. (1979) who further showed that the duration of action of lorazepam is three to four times longer than that of equivalent doses of diazepam. Lorazepam's delayed onset of action and prolonged duration of action as compared with diazepam are somewhat perplexing since lorazepam's half-life (13.2 hours) and volume of distribution (0.84 L/kg (Greenblatt et al., 1977)) are considerably less than the values reported for diazepam. Furthermore, unlike diazepam, lorazepam does not have an active metabolite, since lorazepam is rapidly converted to its major metabolite, lorazepam glucuronide (Elliott, 1976).

Ameer and Greenblatt (1981) have commented on the somewhat paradoxical clinical profile of lorazepam and concluded that, "because the lipophilicity of lorazepam is much less than that of the prototype benzodiazepine, diazepam, the onset of clinical activity of intravenous lorazepam following a single dose is much slower than that of diazepam. This effect can be attributed to the slower rate of loraze-

pam's passage across the blood brain barrier" (p.162). These authors further suggest that "since the extent of tissue distribution of lorazepam also is less than that of diazepam, single doses of lorazepam would appear to have a longer duration of action than will single doses of diazepam" (p.162).

Unfortunately, lorazepam, like diazepam, is not water soluble and is prepared for injection in a propylene glycol solution. However, although there are reports of thrombosis and phlebitis after intravenous injection of lorazepam, these complications are seen less frequently than following diazepam injections (Hegarty and Dundee, 1977; Dundee et al., 1979). The chemical structure of lorazepam, which is similar to that of diazepam is:



Cardiovascular and Respiratory Effects

Only minimal cardiovascular and respiratory effects have been seen following the administration of lorazepam. Alps et al. (1973) found that doses of lorazepam that were three times those used therapeutically produced only slight changes in heart rate, blood pressure and myocardial contractile force. However, at doses significantly above therapeutic doses, some depression in heart rate and blood pressure were reported. Knapp and Fierro (1974) also evaluated the cardiopulmonary safety of lorazepam and found no significant electrocardiographic, hemodynamic or respiratory changes in noncardiac surgery patients receiving lorazepam. Furthermore, Gale and Galloon (1976) used lorazepam as a premedicant in gynecological patients and reported that pulse rate, blood pressure and respiration were remarkably stable in all patients.

In summary, lorazepam is a benzodiazepine that has a prolonged onset and duration of action and clinical effects that are similar to those of diazepam. Because lorazepam causes minimal cardiovascular and respiratory depression, it would appear to be an ideal premedicant in patients with compromised cardiopulmonary function. It would also appear to be a good candidate for further investigation to determine if it might blunt elevations in catecholamines, corti-

sol and renin activity often seen in response to intraoperative stresses.

Midazolam

Chemistry and Pharmacology

Midazolam is an investigational benzodiazepine that resembles both diazepam and lorazepam in its clinical effects. Like these benzodiazepines, midazolam has anxyolytic, anticonvulsant, sleep-inducing, muscle-relaxant and sedative effects, and is currently being tested for induction of anesthsia (Gamble et al., 1981; Pieri et al., 1981; Schwander and Sansano, 1981). Although midazolam resembles diazepam and lorazepam in its therapeutic actions, midazolam does have some interesting characteristics that differentiate it from the other benzodiazepines.

Like diazepam, midazolam has a rapid onset of action with sleep occurring from 1-1/2 to 3 minutes following its intravenous administration (Reves et al., 1978; Jones et al., 1979; Gamble et al., 1981). Midazolam is reported to be twice as potent as diazepam (Dundee, 1980). Unlike both diazepam and lorazepam, the clinical effects of midazolam are of short duration, with drowsiness lasting for approxi-

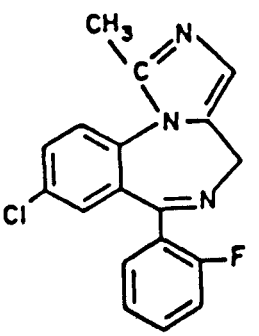
mately two hours and mental clouding for approximately one hour (Forster et al., 1980).

The time course of the clinical effects of midazolam correlates well with its pharmacokinetic profile. Following an intravenous injection of midazolam, the drug is rapidly and widely distributed with a volume of distribution of 1.14 L/kg (Smith et al., 1981). In contrast to most benzodiazepines, midazolam shows a relatively short elimination half-life of 1.77-2.5 hours and is rapidly cleared from plasma at 6.38 ml/min/kg. It is similar to other benzodiazepines in that it is extensively (94%) bound to plasma proteins (Smith et al., 1981; Allonen et al., 1982). The short duration of midazolam's clinical effects may be related not only to the short elimination half-life and large volume of distribution, but also to the biological inactivity of midazolam's metabolites. These metabolites are rapidly formed from midazolam by hydroxylation and subsequent conjugation with glucuronic acid (Heizmann and Ziegler, 1981; Vree et al., 1981).

The pharmacokinetic profile of midazolam, when contrasted with that of diazepam and lorazepam, shows midazolam to be a unique benzodiazepine. Midazolam resembles diazepam in having a fast onset of action, but differs from both diazepam and lorazepam in having a short duration of action.

The elimination half-life of midazolam is considerably shorter than that of either diazepam or lorazepam, the extent of plasma protein binding is intermediate between that reported for diazepam and lorazepam, and the volume of distribution and rate of clearance are higher than the values reported for either diazepam or lorazepam. However, despite all the pharmacokinetic differences in these three benzodiazepines, their clinical effects are quite similar.

In addition to the pharmacological differences between midazolam and the other benzodiazepines used in this study, midazolam has some unique chemical properties. From the following chemical structure of midazolam, it can be seen that midazolam, unlike diazepam and lorazepam, is an imidazole benzodiazepine:



Midazolam exhibits a pH-dependent opening of the benzodiazepine ring. At a pH less than 4.0 the ring opens reversibly producing a water-soluble compound. However, at a more physiological pH, the ring closes and the compound becomes lipid rather than water soluble (Dundee, 1979). Midazolam does not require a propylene glycol vehicle. Consequently, injections of midazolam rarely result in pain on injection or venous irritation (Fragen et al., 1978; Dundee et al., 1980; Gamble et al., 1981).

Cardiovascular and Respiratory Effects

For the most part, midazolam has been shown to have only modest cardiopulmonary actions. The most frequently reported hemodynamic actions of midazolam in human volunteers and experimental animals are a slight decrease in mean arterial pressure and an increase in heart rate (Reves et al., 1978; Jones et al., 1979; Forster et al., 1980). Although many investigators have found respiration remains relatively stable following midazolam injections, brief episodes of apnea have been reported (Reves et al., 1978; Forster et al., 1980).

Midazolam has been administered to patients undergoing cardiac surgery (Reves et al., 1979; Samuelson et al., 1981; Morel et al., 1981). In all of these studies, midazolam was used for induction of anesthesia. The authors reported slight, but clinically acceptable, cardiovascular and respiratory depression, and concluded that midazolam was safe and acceptable for use in patients with cardiac dysfunction.

Midazolam is an imidazole benzodiazepine with a fast onset of action, relatively short duration of action, lack of pain on injection and minimal cardiovascular and respiratory effects. Midazolam, when finally released for general use by the Food and Drug Administration, would appear to have widespread use in anesthesia and surgical patients. A study of its effects on plasma catechoalmines, cortisol and renin activity during hypotensive and surgical stresses is indicated.

Effects of Benzodiazepines on Plasma Catecholamines

Over the past decade Zsigmond and his associates have reported that diazepam is effective in preventing the cardiovascular stimulation caused by ketamine anesthesia (Zsigmond et al., 1974; Kothary et al., 1975; Kumar et al., 1978; Kumar et al., 1980; Zsigmond et al., 1980). In this series of studies, involving both cardiac and noncardiac surgery patients, ketamine administered in the absence of diazepam resulted in tachycardia, hypertension and increased sympathetic activity. However, when diazepam was administered prior to ketamine, the circulatory side effects of ketamine were absent and no significant elevations were seen in either epinephrine or norepinephrine levels.

Further beneficial effects of diazepam were shown by Melsom et al. (1976). In this study, patients admitted to the coronary care unit with acute myocardial infarction were divided into a diazepam group and a control group. Patients treated with diazepam showed a marked decrease in urinary catecholamines compared with patients not given diazepam. These authors also reported that the incidence of severe cardiac arrhythmias was less frequent in the diazepam group.

Additionally, Hoar et al. (1981) showed that diazepam may reverse anesthesia-induced elevations in plasma cate-

cholamines. The authors showed that significant elevations in plasma epinephrine and norepinephrine normally seen following morphine anesthesia were reversed following the administration of diazepam.

The effects of diazepam on catecholamines during surgical stress are difficult to ascertain from the studies reported in the literature. In the Hoar et al. (1981) study, diazepam reversed morphine-induced elevations in catecholamines; however, following surgical stimulation the catecholamines rose to levels well above baseline levels. Since a diazepam-free control group was not included in this study, it cannot be determined whether the postsurgical levels would have been even greater in the absence of diazepam. However, this is probably the case since the postsurgical catecholamine levels were well below the postmorphine levels, and anesthesia is generally considered to be a milder stress than surgery.

Other studies have reported significant elevations in plasma catecholamines following diazepam. Significant elevations in plasma catecholamines during the cardiopulmonary bypass period were seen in patients premedicated with diazepam followed by enflurane-nitrous oxide anestheisa (Tan et al., 1978), morphine-nitrous oxide anesthesia (Balasaraswathi et al., 1978) or fentanyl-oxygen anesthesia (Lappas et

al., 1981). Furthermore, Reves et al. (1982) found that diazepam given prior to the onset of cardiopulmonary bypass failed to attenuate subsequent significant elevations in catecholamines. However, all of these studies, like the Hoar study, lacked a diazepam-free control group. Also, a variety of premedications were given to the patients in these studies that could have influenced the catecholamines independent of surgical and bypass stresses. Diazepam can attenuate anesthesia-induced elevations in catecholamines; however, the effects of diazepam on surgery-induced elevations in catecholamines are not well defined.

Effects of Benzodiazepines on Plasma Cortisol Levels

Fewer studies have been conducted on the effects of benzodiazepines on plasma cortisol and renin activity than their effects on plasma catecholamines. However, there are some indications that benzodiazepines can counteract stress-induced elevations in cortisol. James and Fisher (1970) premedicated gynecological patients with nitrazepam, a benzodiazepine structurally similar to diazepam, and found plasma cortisol levels significantly reduced in response to surgical stress. A similar reduction in plasma cortisol

levels was later found by Wesseling and Edens (1975) in patients undergoing broncoscopy who had been premedicated with a relatively high dose of diazepam. Moreover, diazepam, as well as other benzodiazepines not used in clinical anesthesia such as chlordiazepoxide, clorazepate and clonazepam were effective in reducing stress-induced elevations in plasma corticosteriod levels in rats (Le Fur et al., 1979). On the other hand, in the previously discussed Melsom et al. study (1976) in which diazepam was shown to reduce urinary catecholamine excretion in patients with acute myocardial infarction, no effect of diazepam on plasma cortisol levels was noted. However, in this study, cortisol levels were not elevated in the patients who did not receive diazepam. Thus, there is evidence to suggest that when stress-induced elevations in cortisol occur, these rises can be attenuated by benzodiazepine pretreatment.

Effects of Benzodiazepines on Plasma Renin Activity

The role of diazepam in counteracting stress-induced elevations with regard to plasma renin activity has not been widely studied. Lappas et al. (1981) found that cardiac surgery patients had elevations in plasma renin activity

following fentanyl-diazepam anesthesia and these elevations were maintained following surgical stimulation. These results suggest that diazepam is ineffective in counteracting a stress-induced increase in plasma renin activity. However, because there was no drug-free control group it is difficult to interpret these results. Although diazepam does not appear to attenuate plasma renin activity, the precise role of diazepam in plasma renin activity has not been determined.

Summary

Diazepam has been shown to attenuate, but not abolish, stress-induced elevations in plasma catecholamines and cortisol. There are no reports of a benzodiazepine attenuation of plasma renin activity. Because a diazepam-free control group was not used in many studies, the results are difficult to interpret and additional studies are needed to clarify confusing data.

The Purpose of This Study

The present animal study will investigate three benzodiazepines that are used in clinical anesthesia. Their effects on plasma catecholamines, cortisol and renin activity following drug-induced hypotension and surgical trauma will be determined. More specifically, this study will:

1. Stress an anesthetized dog by drug-induced hypotension or surgical trauma and then simultaneously examine changes in hemodynamic variables (MAP, HR, CI, RAP, SVRI, Wedge, LVSWI - see Methods for detailed explanation of these hemodynamic variables) and changes in plasma catecholamines, cortisol and renin activity.

2. Compare the abilities of diazepam, lorazepam, and the investigational benzodiazepine midazolam, to attenuate hypotension-induced elevations in plasma catecholamines, cortisol, and renin activity.

3. Investigate the ability of midazolam to attenuate surgery-induced elevations in plasma catecholamines, cortisol and renin activity.

4. Examine the ability of midazolam to attentuate hypotension-induced elevations in plasma catecholamines and cortisol over a time course similar to that seen in many clinical situations.

This study will provide the knowledge of how diazepam, lorazepam, and midazolam influence hormonal responses in animals subjected to hypotensive and surgical stresses. It is anticipated that these results will provide a basis for future patient studies involving these drugs.

CHAPTER II

METHODS

General Procedures

Subjects

Male mongrel dogs weighing 13-18 kg were used in this study, which was approved by the Institutional Committee on Animal Care. The animals were individually housed and cared for at the Loyola University Animal Research Facility. Except for Experiment III, each animal was used in two experiments. After participating in one experiment, the animals were returned to the animal research facility and allowed to recuperate before being used a second time. The dogs were killed while anesthetized with a lethal injection of potassium chloride upon completion of the second experiment.

Procedures

In order to minimize presurgical stress due to transport, the dogs were brought to the laboratory one hour prior to surgery. The dogs were not exposed to the laboratory or surgical team prior to their first experimental session. Unconsciousness was induced with a 2% solution of sodium thiopental (*Pentothal*), 20.0 mg/kg, which was injected into a *Butterfly* catheter placed in the cephalic vein. Following induction, the dogs were intubated using a cuffed endotracheal tube and mechanically ventilated with 100% oxygen. Anesthesia was maintained at 1.3 MAC (MAC refers to the minimum alveolar concentration of an anesthetic necessary to prevent movement to skin incision in 50% of subjects) using nitrous oxide-oxygen (1:2) and 2% enflurane (*Ethrane*). Depth of anesthesia was estimated by monitoring standard clinical signs of anesthesia such as respiratory pattern, eyelid and conjunctival reflexes, pupil size, blood pressure and heart rate (Cohen, 1975). Blood anesthesia levels were periodically measured using gas chromatography. Standard electrocardiogram (ECG) needle electrodes were positioned on the dogs so that the Lead II ECG could be continuously monitored on an oscilloscope.

Percutaneous cannulas were placed in both femoral arteries. Arterial blood pressure was continuously measured via one of the cannulas, and arterial blood samples were drawn from the other cannula. A urinary catheter was inserted so that urine output could be monitored. To maintain hydration, lactated Ringer's solution was dripped through the *Butterfly* cannula at a rate of 5 drops/minute.

Cardiac and pulmonary hemodynamics were monitored using a dog *Swan-Ganz* catheter (Edwards Laboratories No. 93A-095-7F). The frequency of the commercially purchased arterial, central venous and pulmonary artery pressure lines was measured in this laboratory, and each was recorded as 25 Hz. The *Swan-Ganz* catheter was inserted into the right external jugular vein with the aid of a *Cordis* introducer. The balloon-inflated catheter tip was advanced through the right atrium, and right ventricle and advanced until it wedged in a branch of the pulmonary artery. Correct placement of the catheter tip was confirmed from pulmonary artery pressure tracings. A thermistor near the tip of the *Swan-Ganz* catheter was used to measure pulmonary artery blood temperature.

Arterial blood samples were drawn throughout the experiment to monitor blood gases. Blood gases including pH, arterial oxygen tension and arterial carbon dioxide were

measured using a No.165 Corning pH Blood Gas Analyzer, and hematocrit was measured using micro hematocrit capillary tubes. Respiration was adjusted to maintain arterial carbon dioxide at 35-40 mm Hg and pH at approximately 7.4.

After the experimental set-up was completed and the dog's heart rate, blood pressure and blood carbon dioxide levels were stable, the dog was maintained on anesthesia for one hour. Following this one-hour period, the hypotensive or surgical stress was initiated.

Experimental Design

This study, which was conducted in enflurane-nitrous oxide anesthetized dogs, consisted of three experiments. The first experiment was undertaken to investigate changes in plasma epinephrine, norepinephrine, cortisol and renin activity resulting from nitroprusside-induced hypotension in anesthetized dogs. The effectiveness of acute intravenous injections of diazepam, lorazepam or midazolam in inhibiting these responses was also investigated in this experiment.

The second experiment investigated the ability of midazolam to block the hypotension-induced increases in plasma epinephrine, norepinephrine and cortisol when midaz-

olam was administered prior to the administration of anesthesia.

The third experiment consisted of two parts. In the first part, changes in plasma epinephrine, norepinephrine, cortisol and renin activity which resulted from a thoracotomy and rib spread were determined. In the second part, the ability of an acute injection of midazolam to blunt these responses was investigated.

Forty-two dogs were used in this study. Four groups of six dogs were used in the first experiment, one group of six was used in the second experiment and two groups of six were used in the third experiment.

The experimental sessions began at approximately 10:00 a.m. The animals were stablized on anesthesia by 11:00 a.m. The drug-induced hypotension or thoracotomy procedures were then initiated at approximately 12:00 p.m.

Experiment I. Effects of Diazepam, Lorazepam and Midazolam on Nitroprusside-Induced Elevations in Plasma Epinephrine, Norepinephrine, Cortisol and Renin Activity

Experiment I(a). In Experiment I(a) the effects of nitroprusside-induced hypotension on plasma catecholamines, cortisol and renin activity were determined. The results of this experiment then served as the control with which the results from Experiments I(b), I(c), I(d) and II could be compared.

The general procedures outlined above were followed. After the one hour period of anesthesia, mean arterial blood pressure was dropped by 30% by infusing 0.01% sodium nitroprusside (*Nipride*) into the cephalic vein. Blood pressure was stabilized at this lower level (usually within 7 minutes), and the hypotension was continued for 20 minutes. The nitroprusside infusion was then terminated and blood pressure allowed to return to prestress levels.

In order to measure plasma catecholamines, cortisol and renin activity, 18-ml blood samples were drawn at the following intervals: (1) immediately before the hypotensive stress (prestress control); (2-4) 2, 10 and 20 minutes after stable hypotension was achieved; and (5) 20 minutes after

blood pressure returned to prestress levels following the offset of the hypotensive stress. Thus, a total of approximately 90 mls of blood was drawn during each experimental session for subsequent assay. Each blood volume removed was replaced with saline. In all experiments, the blood was immediately placed in an ice bath and then processed according to assay procedures (see p. 53-57) or frozen for later assay. Hemodynamic measurements including SAP, DAP, MAP, PCWP, CO and HR (see pages 58-63 for a complete description) were also taken at intervals 1-5.

Experiment I(b). In Experiment I(b) the effects of diazepam on the responses to a hypotensive stress were examined. The procedure was the same as that described in Experiment I(a), except that diazepam, 0.4 mg/kg i.v., was administered following the withdrawal of the prestress control blood sample.

Experiment I(c). In experiment I(c) the effects of midazolam on the response to a hypotensive stress were examined. The procedure was the same as that described in Experiment I(b), except that midazolam, 0.2 mg/kg i.v., was administered instead of diazepam.

Experiment I(d). In experiment I(d) the effects of lorazepam, a benzodiazepine with a prolonged onset of

action, on the responses to a hypotensive stress were examined. The procedure was the same as that described in Experiment I(b), except that because of the delayed onset of action of lorazepam, the nitroprusside infusion was started 30 minutes after the lorazepam (.07 mg/kg i.v.) injection.

Experiment II. The Effects of Midazolam Given 1-1/2 Hours Prior to the Onset of a Hypotensive Stress on Plasma Concentrations of Epinephrine, Norepinephrine and Cortisol.

In this experiment, midazolam, 0.2 mg/kg iv., was slowly administered to awake, unanesthetized animals. This procedure was done to determine whether the effects of midazolam on the responses to a hypotensive sress would continue over a time span similar to that of many clinical procedures. The protocol outlined in Experiment I(a) was followed, except that midazolam was administered 10 minutes before the induction of anesthesia. Because the results of Experiment I indicated that midazolam does not block hypotension-induced elevations in plasma renin activity, the plasma in this experiment was assayed only for catecholamine and cortisol levels. The catecholamine and cortisol data obtained from Experiment I(a) served as a basis for comparison.

Experiment III. The Effects of Midazolam on Plasma Epinephrine, Norepinephrine, Cortisol and Renin Activity Following Surgical Trauma

In Experiment III(a) the effects of surgical stress on plasma catecholamines, cortisol and renin activity were investigated. The results from this experiment then served as the control with which the results from Experiment III(b) could be compared.

Experiment III(a). In this experiment the general procedures were the same as previously described. However, the animals in this experiment were subjected to a surgical rather than hypotensive stress. After one hour of stable anesthesia, thoracotomies were performed on the dogs. The thoracotomies were performed by first making a skin incision on the right side of the chest to expose the 5th and 6th ribs. The pleural cavity was then entered between these ribs and the intrarib distance at peak inspiration measured. A self-maintaining retractor was placed between the 5th and 6th ribs at a position 90-110 mm from the sternal midline and spread so that the distance between these ribs was 4 times the intrarib distance, ranging 65 to 75 mm. (This distance was found to be the maximum rib spread that occur-

red without rib breakage.) Final placement of the retractor took approximately 5 minutes from skin incision. The retractor remained in place for 20 minutes and then was removed.

Blood samples for chemical assays (approximately 18 mls) were drawn from the femoral artery cannula at the following intervals: (1) immediately before skin incision (prestress control); (2-5), 2, 5, 10 and 20 minutes after placement of the retractor; and (6) 20 minutes after removal of the retractor. Hemodynamic measurements were also taken at sample intervals 1-5, and heart rate and blood pressure were measured at one minute intervals from the time of skin incision to placement of the retractor.

Experiment III(b). In Experiment III(b), the effects of midazolam on the stimulatory response to surgical trauma were investigated. The procedure described for Experiment III(a) was followed, except that 2 minutes prior to skin incision, midazolam, 0.2 mg/kg i.v., was injected into the cephalic vein.

Biochemical Determinations

Catecholamine Fluorometric Assay

Plasma epinephrine and norepinephrine concentrations were determined using a modification of the method of Vendsalu (1960) and Haggendal (1963). Thirteen milliliters of the total arterial blood withdrawn at each time period were collected in heparinized centrifuge tubes for this assay. The heparinized blood was centrifuged for 10 minutes at 4,000 RPM at a temperature of +4°C. The plasma was then removed, measured for volume, and approximately 0.1 ml of a solution containing 10% EDTA and 2% ascorbic acid in 4.0 N perchloric acid was added to every ml of plasma to deproteinize the plasma and stabilize the catecholamines. The treated plasma was refrigerated for 30 minutes at +6°C and then centrifuged for 20 minutes. The acidified supernatant was collected and the protein pellet re-extracted with 0.4 N PCA. After centrifugation the two supernatants were combined. The acidified deproteinized plasma containing epinephrine and norepinephrine were then stored at -20°C.

Purification and isolation of epinephrine and norepinephrine were accomplished using ion-exchange column chroma-

tography as described by Glisson (1971). This technique involved thawing the acidified deproteinized plasma and then passing the plasma sample through an ion exchange column buffered to a pH of 6.5. The resin column was washed with water followed by 1.5N hydrochloric acid to elute epinephrine and norepinephrine from the resin column. The acid eluate, containing epinephrine and norepinephrine, was collected and stored at -20°C until the samples were thawed for further processing (<48 hrs.). Epinephrine and norepinephrine were quantified upon conversion of the amines to their respective fluorophores using the trihydroxyindole technique (Glisson et al., 1972). Developed fluorescence was then measured at 405/505 and 455/505 nM in and Aminco-Bowman spectrophotofluorometer equipped with an ellipsoidal mirror condensing system. Plasma epinephrine and norepinephrine concentrations were calculated from a standard curve and expressed as ng/ml (cf., Appendix B). Maximum sensitivity of this system was determined to be plus or minus .45 ng/ml. Using a minimum 5.0 ml plasma sample provided sufficient concentration to quantify. Approximately 80% recovery of epinephrine and norepinephrine have been found in this laboratory. Reported values are uncorrected. The between-assay coefficient of variation was approximately 10%.

Renin Activity Radioimmunoassay

Plasma renin activity was assayed in duplicate using the Becton Dickinson Renin Activity Radioimmunoassay Kit (^{125}I), as modified from the method of Haber et al. (1969). According to this procedure, 5-ml samples of arterial blood were collected in prechilled *Vacutainer* tubes containing sufficient disodium EDTA to achieve a final concentration of 1.4 mg/ml. The plasma was immediately separated in a refrigerated centrifuge. One milliliter of the cold plasma was then transferred to a polystyrene test tube and stored at -20°C until the radioimmunoassay was performed. The remainder of the plasma was also stored at -20°C for later use in the cortisol radioimmunoassay.

To perform the radioimmunoassay, the plasma was thawed and buffered by Tris Chloride to pH 7.4. Dimercaprol and 8-hydroxyquinoline solution were added to the buffered plasma to inhibit the transformation of angiotensin I to angiotensin II and breakdown products. Angiotensin I was then generated by placing treated plasma in a heated bath (37°C) for 3 hours. A blank was made by stimultaneously storing the other half of the treated plasma in an ice bath.

Following Angiotensin I generation, the assay procedure was initiated. The assay involved adding a constant

amount of angiotensin ^{125}I tracer and angiotensin antiserum in tris acetate buffer to test tubes containing either the plasma samples or angiotensin I standards. The tubes were then incubated 20-24 hours at 2-8°C. After incubation, separation of the bound and free angiotensin was achieved rapidly by contact with dextran-coated charcoal. The antibody-bound ^{125}I in the supernatant solution was then decanted into a numbered tube and counted using a Packard Liquid Scintillation Spectrometer adjusted for the measurement of ^{125}I. A standard curve was plotted and the concentration of angiotensin I in the plasma was determined from the standard curve and expressed as ng/ml/hr of angiotensin I generated (cf., Appendix B). The sensitivity of this assay was shown to be 0.14 ng angiotensin I/ml plasma, and the precision of the assay was shown by a within-assay coefficient of variation (C.V.) of 2.9% and between-assay C.V. of 8.5%

Cortisol Radioimmunoassay

Plasma cortisol concentrations were measured in duplicate using the *Amerlex* Cortisol RIA Kit. The plasma samples used in this assay were collected according to the procedures described for the renin activity radioimmunoassay.

In this assay, cortisol ^{125}I and cortisol antiserum were pipetted into tubes containing 50 mcl aliquots of the standard, control or experimental serum samples. The tubes were incubated in a 37°C water bath for one hour and centrifuged for 15 minutes. The supernatant was counted in a Packard Liquid Scintillation Spectrometer. Finally, a standard curve was constructed, and the concentration of cortisol in the plasma was determined from the curve and expressed as mcg cortisol/100 ml plasma (cf., Appendix B). The sensitivity of this assay was shown to be 0.1 mcg cortisol/100 ml plasma, and the precision of the assay was shown by a within-assay C.V. of 5.7% and between-assay C.V. of 8.9%.

Hemodynamic Monitoring

Hemodynamic monitoring provides direct measurement of cardiac performance. From these measurements, derived indices can be calculated. The raw and derived data then were used to evaluate cardiovascular function.

Direct Physiological Measurements

Cardiovascular Pressures

The femoral artery catheter was used to measure systolic arterial pressure (SAP) and diastolic arterial pressure (DAP). From these pressures the mean arterial pressure (MAP) was calculated as:

$$MAP = (DAP + 1/3(SAP-DAP))$$

The mean arterial pressure is the average pressure during a given cardiac cycle that exists in the aorta and its major branches (Berne and Levy, 1977). Mean arterial pressure is the average pressure tending to push blood through the systemic circulatory system (Guyton, 1976). This, as well as other cardiovascular pressures, are expressed in mm Hg.

The *Swan-Ganz* cathether was used to obtain pulmonary capillary wedge pressure (PCWP or wedge), pulmonary artery systolic pressure (PASP), pulmonary artery diastolic pressure (PADP) and right atrial pressure (RAP). The wedge pressure is the most useful of these pressures because it reflects left ventricular end diastolic pressure (Gilbert and Hew, 1979; Kaplan, 1979). Consequently, an elevated wedge pressure usually indicates an acute fluid overload or a decrease in myocardial performance (Roizen et al., 1981). Right atrial pressure is used to evaluate right ventricular function. The pulmonary pressures provide data on pulmonary vascular resistance.

Cardiac Output

Cardiac output (CO) is the volume of blood pumped by the left ventricle into the aorta per unit time, typically per minute. Under normal circumstances, the major factor determining the cardiac output is venous return, which is the amount of blood that is pumped into the heart each minute. When the arterial pressure remains normal and the heart is healthy, cardiac output is controlled by the needs of local tissues throughout the body (Guyton, 1976). It should be noted that during anesthesia, control of cardiac output circulatory dynamics differs from the non-anesthetic

state, reflecting a balance of depressant actions by anesthetic agents and compensating reflexes (Hickey and Eger, 1981, Kaplan, 1981).

Two major determinants of venous return and cardiac output are arterial pressure and total peripheral resistance (Guyton, 1976). Arterial pressure is controlled by three groups of mechanisms that differ in their onset of action. The first group consists of short term mechanisms including (1) the hormonal norepinephrine - epinephrine vasoconstrictor mechanism, and (2) nervous mechanisms such as the baroreceptor mechanism, the chemoreceptor mechanism and the CNS ischemic feedback mechanism.

The baroreceptor mechanism is probably the best known of the short-term nervous mechanisms for regulating arterial pressure (Guyton, 1976). Baroreceptors are nerve endings lying in the walls of arteries and are especially abundant in the walls of the internal carotid arteries and the walls of the aortic arch. Baroreceptors are stimulated when stretched and respond rapidly to changes in arterial pressure. They respond much more to a rising pressure than a falling pressure. Impulses from the baroreceptors inhibit the vasoconstrictor center of the medulla and excite the vagal center. The net effects, which are mediated through sympathetic and parasympathetic nerves, are vasodilation

throughout the peripheral circulation and decreased cardiac output and strength of contraction.

The short-term mechanisms begin to act within seconds whenever any disturbance attempts to change the arterial pressure to a value higher or lower than its normal level. Baroreceptors are probably of no importance in long-term regulation of arterial pressure since they adapt in one to two days to whatever pressure level they are exposed.

The intermediate-term pressure control mechanisms are represented by stress-relaxation of the vasculature, the renin-angiotensin-vasoconstrictor mechanisms and the capillary fluid shift mechanism. These intermediate pressure control mechanisms become activated when a disturbance which tends to alter arterial pressure continues for minutes or hours.

The long-term arterial pressure control mechanisms are represented by the renal-body fluid pressure control system and the aldosterone control system. These long term pressure control mechanisms are slow to act, usually requiring that the disturbance altering arterial pressure continue for hours before they become effective.

The second major determinant of cardiac output is total peripheral resistance, also known as systemic vascular resistance. Resistance refers to the impediment to blood

flow in a vessel, and total peripheral resistance is the resistance of the entire systemic circulation. The resistance of a vessel is directly proportional to the blood viscosity and length of the vessel, but inversely proportional to the fourth power of the radius of the vessel (Guyton, 1976).

In this study, cardiac output was measured by thermodilution using a *Swan-Ganz* catheter and an Edwards Laboratory cardiac output computer. The method involved injecting a 3-ml 0°C 5% dextrose-in-water solution into the catheter. The cold solution then flowed into the right atrium, underwent rapid mixing, and the resultant temperature change was detected by a thermistor that was positioned near the tip of the *Swan-Ganz* cathether. Cardiac output, which is inversely proportional to the integral temperature change, was calculated by the cardiac output computer and expressed in L/min. Cardiac output was taken as the mean of three sequential cardiac output measurements within 10% of each other. Cardiac output measurements obtained with the *Swan-Ganz* catheter were previously shown in this laboratory to correlate well with cardiac measurements obtained using indocyanine (cardiogreen).

Heart Rate

Heart rate (HR), expressed in beats/minute, was measured using a standard Lead II electrocardiogram (ECG). The central and autonomic nervous systems are the main factors affecting heart rate (Kaplan, 1979); that is, heart rate is increased by sympathetic activity and parasympathetic withdrawal, and decreased by parasympathetic activity.

Derived Indices

Using directly measured hemodynamic data, parameters can be calculated that provide important additional insights into cardiovascular function. In order to compare the hemodynamic profiles of animals of different body sizes, the variables are corrected by dividing by the body surface area (BSA). Such corrected parameters are expressed as indices.

Cardiac Index

Cardiac index (CI), expressed in $L*min^{-1}*M^{-2}$, was calculated as:

$$CI = (CO/BSA)$$

Systemic Vascular Resistance Index

Systemic vascular resistance index (SVRI) is a measure of peripheral vascular constriction of the small arteries or arterioles. It is an indicator of the resistance that the heart must overcome to pump blood through the systemic circulation. Vasodilator drugs such as nitroprusside can decrease the systemic vascular resistance. Catecholamines have differential effects on systemic vascular resistance; that is, norepinephrine tends to increase it, isoproterenol tends to decrease it, and the effects of epinephrine vary with dosage and target organ (Innes and Nickerson, 1975). Systemic vascular resistance index, expressed in dyne*sec*cm^{-5}*M^2, was calculated as:

$$SVRI = (((MAP-RAP)*79.9)/CI)$$

Left Ventricular Stroke Work Index

Left ventricular stroke work index (LVSWI or stroke work index) is a measure of the amount of work performed by the heart. A depressed stroke work index suggests that the pumping ability of the heart is weakened. An elevated stroke work index, on the other hand, may reflect a compensatory mechanism for a condition requiring an abnormally high contractile force (Brantigan, 1982). An elevated

stroke work index is also an indication that an increased oxygen demand is being placed on the heart. Stroke work index depends in part on stroke index (SI). Stroke index is the amount of blood pumped per heartbeat, expressed in $ml*M^{-2}$, and calculated as:

$$SI = (CI/HR)$$

Stroke work index, in $g*M*M^{-2}$, is then calculated as:

$$LVSWI = ((13.6(MAP-PCWP))*SI)$$

Although all of the hemodynamic variables which have been described were collected during the course of the experiments, only selected variables exhibiting noteworthy changes will be presented and discussed throughout this paper.

Data Analysis

The data were analyzed using an IBM 3033-S computer system and the Statistical Analysis System (Helwig and Council, 1979) computing package. Initially a split-plot analysis of variance (ANOVA) was performed on the data. When significant results were obtained from the ANOVA, further testing was done. The effects of stress within individual drug groups was assessed using Duncan's new multiple range test. A comparison of the effects of different drugs at a given time period was made using the nonpaired Student's *t*-test. In all experiments in the present study, a probability of less than .05 was considered statistically significant.

The Analysis of Variance

Indications

When an experiment involves more than two treatment means, the first statistical test should be an ANOVA to rule out the possibility that any observed differences between means are merely due to sampling fluctuations (Wallenstein

et al., 1980; Zivin and Bartko, 1976; Arkin and Colton, 1970). If the ANOVA indicates that differences probably exist, then the investigator may proceed with further testing to determine which pairs of means differ from each other.

Basic Concepts

The ANOVA tests the hypothesis that all treatment means are equal. In its simplest form, ANOVA accomplishes this by comparing two different estimates of population variances. (Variance is simply the square of a standard deviation.) One variance, based on differences among the treatment means, is referred to as the ***between-group variance***. The other variance, based on the variation of values within each treatment is called the ***within-group variance***. If the initial hypothesis is correct and all treatment means are equal, then these two estimates of the population variance should be similar. In fact, they should differ only by an amount equal to that which might arise from sampling fluctuations. However, if the between-group variance is significantly greater than the within-group variance, then the differences among the treatment means are not due to sampling fluctuations alone, but rather reflect a true difference.

The *F*-test is used to compare the between-group and within-group variances. In this test, the variances are first corrected for differences in degrees of freedom by dividing the between-group and within-group variances by their respective degrees of freedom. The calculated values are then referred to as mean square between group (MSBG) and mean square within group (MSWG). The *F*-ratio (the ratio of two independent chi-square variables, each divided by its degrees of freedom) is then calculated as:

$$F = (MSBG/MSWG)$$

The largest value of *F* that might occur due to sampling fluctuations at a given significance level can be found in a table of *F*-values (see Kirk, 1968). If the calculated *F* exceeds this value, then it may be said that the group means differ significantly.

Assumptions

The ANOVA assumes that the measurements are obtained by random sampling from normally distributed populations of equal variances. According to Kirk (1968), populations showing moderate departures from a normal distribution and having unequal variances will have little effect on the *F*-test. It is most important, however, that the measure-

ments are obtained by random sampling. Failure to meet the assumptions of any statistical test will affect both the significance level and the sensitivity of a test. The ANOVA is considered very robust with respect to most assumptions.

Design

The design used in this study has been referred to as a split-plot design by Kirk (1968) and as a two-factor experiment with repeated measures by Winer (1962). A split-plot design is really a composite of two simpler designs -- one design in which each subject receives only one level of a treatment and a second design in which each subject receives all levels of a treatment. Because each dog received only one drug, while being tested at all time periods, this model is consistent with the description of a split-plot design.

From the analysis of variance, each variable under statistical consideration (such as plasma catecholamine levels) can be tested for a drug effect, a time effect, and a drug*time interaction. In the present experiments, a significant drug effect suggests an overall, nontime-related difference between two or more drug treatment groups (diazepam, midazolam, lorazepam or saline treated animals). A significant time effect suggests an overall, nondrug-re-

lated, difference between two or more time periods. A significant drug*time interaction would suggest that two or more drug groups behaved differently over different time periods and consequently did not respond uniformly over the course of the experiment.

Duncan's New Multiple Range Test

After ANOVA indicates that there are significant differences among various time periods, the next step is to determine which pairs of means are different. Using Duncan's new multiple range test, all pairwise comparisons of means can be simultaneously obtained. The Duncan test, fully described by Kirk (1968), first requires ranking the means according to size and then calculating the differences between the means. For a pair of means to be considered significantly different, the difference between the two means must exceed a value determined using a table of Duncan values (see Kirk, 1968).

A major advantage of Duncan's new multiple range test is that the probability level (alpha) is spread across all pairwise comparisons, thus avoiding the increase in alpha that occurs with multiple paired-*t* comparisons. Also, Dun-

can's test is less conservative than many other tests since it will show a significant difference between means more often than most other acceptable multiple comparison procedures.

Nonpaired Student's *T*-Test

When the ANOVA shows that drug groups differ from each other a nonpaired Student's *t*-Test can be used to determine which groups differ from other drug groups at any given time period. In this test, two means are compared by calculating a *t*-statistic (the *t*-statistic equals the ratio of the difference between the two means divided by the standard error of the difference between the means). When the *t*-statistic exceeds a value determined by the degrees of freedom and specified in a table of *t*-values (see Kirk, 1968), the means are considered statistically different. All comparisons in this study were made using two-tailed tests. That is, the probability level (alpha) was equally divided between the two tails of the *t*-distribution.

CHAPTER III

RESULTS

Experiment I

Hemodynamic Measurements

Control Animals

The hemodynamic response of control animals to the nitroprusside-induced hypotension is shown in Table 1. In this group of animals, blood pressure during the nitroprusside infusion was maintained close to the targeted 30% decrease from the prestress control value. Although a rebound hypertension was not seen at the 20-minute postinfusion recovery period, some animals did show a slight (within 10 mm Hg) and transient increase in blood pressure shortly after the nitroprusside infusion was terminated.

Heart rate remained relatively stable throughout the hypotensive period. Moderate decreases in heart rate were seen after 20 minutes of hypotension and lasted into the recovery period.

Table 1. Hemodynamic profile of control (saline-treated) dogs in response to a nitroprusside-induced 30% decrease in mean arterial blood pressure. Abbreviations used are: MAP, mean arterial blood pressure; HR, heart rate; CI, cardiac index; RAP, right atrial pressure; SVRI, systemic vascular resistance index; Wedge, pulmonary capillary wedge pressure; and LVSWI, left ventricular stroke work index. Values represent means plus or minus SEM. The asterisk indicates a significant difference between the indicated value and its corresponding prestress control value.

TABLE 1

HEMODYNAMIC PROFILE OF CONTROL DOGS: HYPOTENSION

	Prestress Control	Hypotension			20 Min Post-Recovery
		2 Min	10 Min	20 Min	
MAP (mm Hg)	92±4	61±3*	64±4*	63±3*	93±6
HR (min^{-1})	118±6	117±3	116±5	110±4	110±6
CI ($L \cdot min^{-1} \cdot M^{-2}$)	4.2±0.3	3.0±0.3*	3.2±0.3*	2.9±0.2*	3.6±0.3*
RAP (mm Hg)	2.8±0.7	2.4±0.6	2.3±0.5	2.4±0.6	3.1±0.8
SVRI ($dyne \cdot sec \cdot cm^{-5} \cdot M^2$)	1744±169	1623±212	1535±146	1653±127	2008±155*
Wedge (mm Hg)	6.0±0.7	5.2±0.5	5.7±0.5	5.8±0.6	6.2±0.5
LVSWI ($gm \cdot M \cdot M^{-2}$)	41±4	18±1*	22±2*	21±2*	40±5

Cardiac index was significantly decreased throughout the hypotensive period. During the recovery period, cardiac index increased, but remained significantly lower than the prestress control value.

Systemic vascular resistance index decreased slightly and insignificantly during the hypotensive period. The maximal decrease (12%) occurred in the first 10-minutes of the hypotensive period. A statistically significant increase in systemic vascular resistance index was seen during the recovery period; however, this value was not greatly elevated above the prestress control value.

Changes in stroke work index were similar to changes in blood pressure, since significant decreases were seen only during the hypotensive period. During the recovery period, stroke work index increased to the prestress control level.

Right atrial and wedge pressures were within the normal range during the hypotensive and recovery periods.

Benzodiazepine-Treated Animals

The hemodynamic response of the dogs pretreated with diazepam, lorazepam or midazolam prior to the hypotensive stress are shown in Tables 2-4. Prestress hemodynamic

Table 2. Hemodynamic profile of diazepam-treated dogs in response to a nitroprusside-induced 30% decrease in mean arterial blood pressure. Abbreviations used are: MAP, mean arterial blood pressure; HR, heart rate; CI, cardiac index; RAP, right atrial pressure; SVRI, systemic vascular resistance index; Wedge, pulmonary capillary wedge pressure; and LVSWI, left ventricular stroke work index. Values represent means plus or minus SEM. The asterisk indicates a significant difference between the indicated value and its corresponding prestress control value.

TABLE 2

HEMODYNAMIC PROFILE OF DIAZEPAM DOGS: HYPOTENSION

	Prestress Control	Hypotension			20 Min Post-Recovery
		2 Min	10 Min	20 Min	
MAP (mm Hg)	94±4	66±3*	64±3*	68±3*	98±5
HR (min^{-1})	111±6	115±12	114±11	112±9	107±7
CI ($L \cdot min^{-1} \cdot M^{-2}$)	3.7±0.4	3.0±0.3*	3.3±0.3	3.4±0.4	3.3±0.4*
RAP (mm Hg)	2.4±0.8	2.1±0.5	3.1±1.1	2.6±0.8	3.2±1.1
SVRI ($dyne \cdot sec \cdot cm^{-5} \cdot M^2$)	1919±282	1683±252	1417±169*	1617±297*	2183±306
Wedge (mm Hg)	5.2±0.7	5.2±0.7	5.2±0.7	5.2±0.9	5.2±0.6
LVSWI ($gm \cdot M \cdot M^{-2}$)	39±3	21±2*	23±2*	24±2*	38±5

Table 3. Hemodynamic profile of lorazepam-treated dogs in response to a nitroprusside-induced 30% decrease in mean arterial blood pressure. Abbreviations used are: MAP, mean arterial blood pressure; HR, heart rate; CI, cardiac index; RAP, right atrial pressure; SVRI, systemic vascular resistance index; Wedge, pulmonary capillary wedge pressure; and LVSWI, left ventricular stroke work index. Values represent means plus or minus SEM. The asterisk indicates a significant difference between the indicated value and its corresponding prestress control value.

TABLE 3

HEMODYNAMIC PROFILE OF LORAZEPAM DOGS: HYPOTENSION

	Prestress Control	Hypotension			20 Min Post-Recovery
		2 Min	10 Min	20 Min	
MAP (mm Hg)	87±3	62±3*	62±3*	61±3*	90±3
HR (min^{-1})	107±6	117±13	114±12	117±9	100±3
CI ($L \cdot min^{-1} \cdot M^{-2}$)	4.5±0.3	3.8±0.4	3.8±0.5	4.1±0.7	4.0±0.3
RAP (mm Hg)	3.2±0.9	2.9±0.8	3.7±1.1	3.8±1.2	3.7±1.0
SVRI ($dyne \cdot sec \cdot cm^{-5} \cdot M^2$)	1486±64	1332±179	1277±142	1229±195	1769±148
Wedge (mm Hg)	4.9±1.1	5.0±1.0	4.8±0.9	5.8±1.1	6.3±1.4*
LVSWI ($gm \cdot M \cdot M^{-2}$)	49±7	27±5*	28±6*	28±6*	45±5

Table 4. Hemodynamic profile of midazolam-treated dogs in response to a nitroprusside-induced 30% decrease in mean arterial blood pressure. Abbreviations used are: MAP, mean arterial blood pressure; HR, heart rate; CI, cardiac index; RAP, right atrial pressure; SVRI, systemic vascular resistance index; Wedge, pulmonary capillary wedge pressure; and LVSWI, left ventricular stroke work index. Values represent means plus or minus SEM. The asterisk indicates a significant difference between the indicated value and its corresponding prestress control value.

TABLE 4

HEMODYNAMIC PROFILE OF MIDAZOLAM DOGS: HYPOTENSION

	Prestress Control	Hypotension			20 Min Post-Recovery
		2 Min	10 Min	20 Min	
MAP (mm Hg)	96±4	61±4*	62±4*	63±3*	93±4
HR (min^{-1})	114±10	108±9	104±8*	104±9*	98±7*
CI ($L \cdot min^{-1} \cdot M^{-2}$)	5.5±0.7	4.3±0.5*	4.4±0.5*	3.8±0.5*	4.5±0.5*
RAP (mm Hg)	4.0±1.1	3.0±1.1*	3.0±1.1*	3.1±1.1*	4.6±1.0
SVRI ($dyne \cdot sec \cdot cm^{-5} \cdot M^2$)	1358±102	1138±126	1101±109*	1299±87	1628±132*
Wedge (mm Hg)	8.5±1.9	7.3±1.5	6.7±1.3	7.3±1.5	8.2±1.4
LVSWI ($gm \cdot M \cdot M^{-2}$)	58±4	29±5*	32±3*	28±4*	52±4

values in these three drug groups were similiar to the control values. Blood pressure during the hypotensive period was maintained at approximately 30% of the prestress control level in all of these groups. Heart rate remained relatively stable, with only the midazolam-treated animals showing any significant changes, and the decreases seen in these animals were not clinically significant.

Cardiac index was decreased somewhat during hypotension in all benzodiazepine groups, but the magnitude of the response varied between the groups. The greatest decreases in cardiac index were seen in the midazolam group, which showed significant decreases throughout the hypotensive and recovery periods. In the diazepam group, the decreases in cardiac index only reached significance at the 2-minute hypotensive period and the recovery period. The lorazepam group showed the least amount of change in cardiac index, since there were no statistically significant decreases in cardiac index in this group.

Changes in systemic vascular resistance index in the benzodiazepine groups were similar to those reported in the control group, as only slight decreases were seen during the hypotensive and recovery periods. Systemic vascular resistance index appeared to be most stable in the lorazepam group because no significant changes were noted at any time

period in these animals. The diazepam group, like the lorazepam group, did not show a significant elevation in systemic vascular resistance index during the recovery period; however, significant decreases were seen after 10 and 20 minutes of hypotension. Changes in systemic vascular resistance index in the midazolam group were most similar to those seen in the control group since a significant decrease was recorded both during hypotension (at 10 minutes) and after recovery of blood pressure.

In all three benzodiazepine groups, stroke work index followed the same pattern as that shown for the control group. There was a significant decrease in stroke work index during the hypotensive period. This increase was not maintained during the recovery period as stroke work index approached prestress control levels at this time.

Changes in wedge pressure in the benzodiazepine groups were similar to changes in wedge pressure in the control animals. No satistically significant changes in wedge pressure were seen in any benzodiazepine group during the hypotensive period. In the lorazepam group, however, wedge pressure began increasing during the last 10 minutes of the hypotensive period, and this increase became statistically significant during the recovery period.

No significant changes in right atrial pressure were seen in either the diazepam or lorazepam groups. However, significant decreases in the midazolam group were seen throughout the hypotensive period.

ANOVA

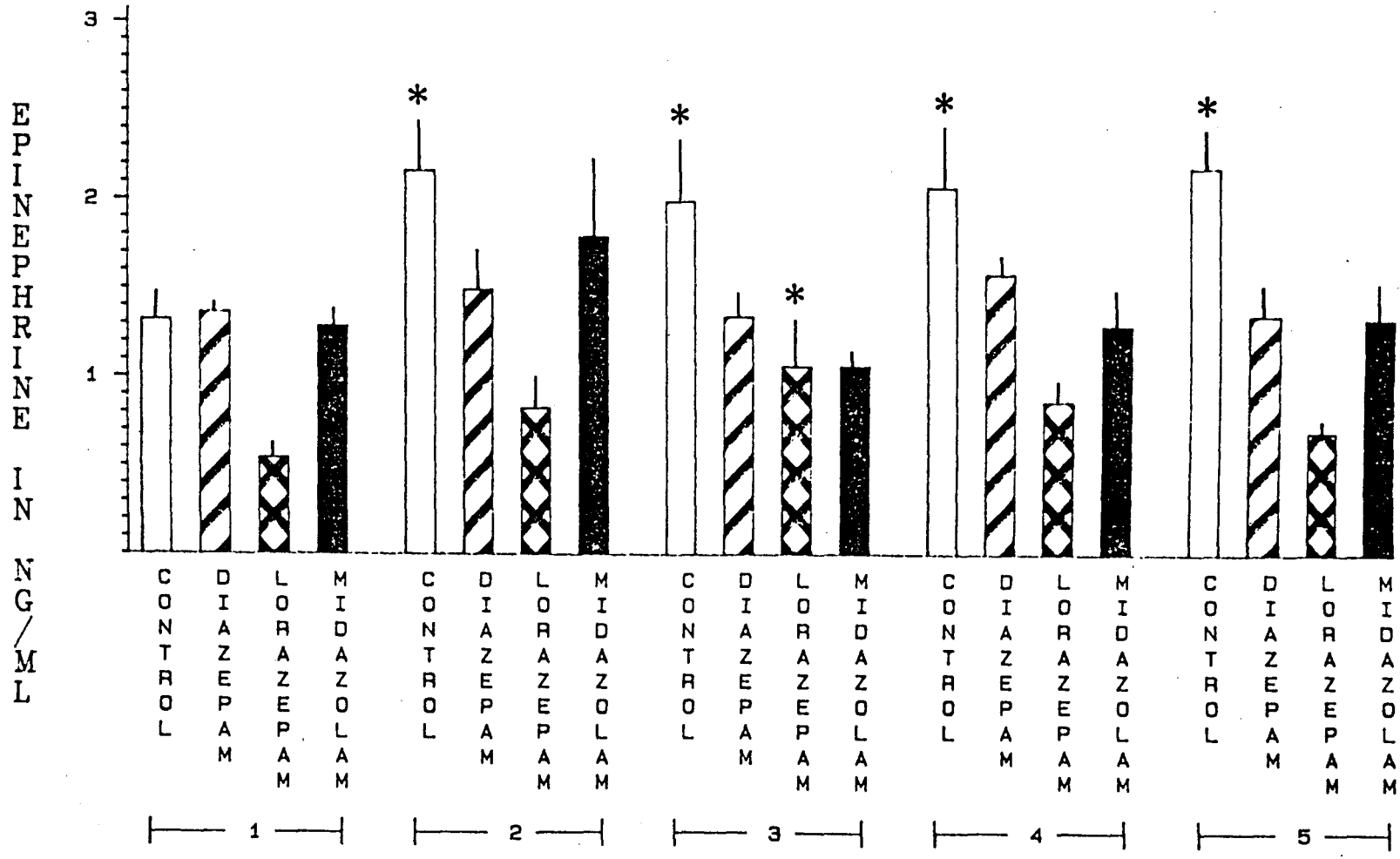
The two-way analysis of variance of the experimental groups showed a significant time effect for all of the hemodynamic variables listed in Tables 1-4. No significant drug effect or drug*time interactions, however, were seen for any hemodynamic variable.

Plasma Catecholamine Concentrations

Control Animals

Epinephrine. Plasma epinephrine levels were significantly elevated throughout the hypotensive period, and these elevated levels were sustained during the recovery period (Figure 1). Within 2 minutes after stable hypotension was achieved, plasma epinephrine levels increased 64% above the prestress control level and then did not change markedly during the remaining hypotensive period or on recovery.

Figure 1. Comparison of plasma epinephrine levels (mean plus or minus SEM) before, during and after a 30% decrease in mean arterial blood pressure in dogs receiving saline, diazepam, lorazepam or midazolam prior to the hypotension. Sample periods are: (1) prior to the hypotension (prestress control); (2-4) 2, 10 and 20 minutes after stable hypotension; and (5) 20 minutes after blood pressure returned to prestress control levels. The asterisk indicates a significant difference ($p<.05$) between the indicated value and its corresponding prestress control value.

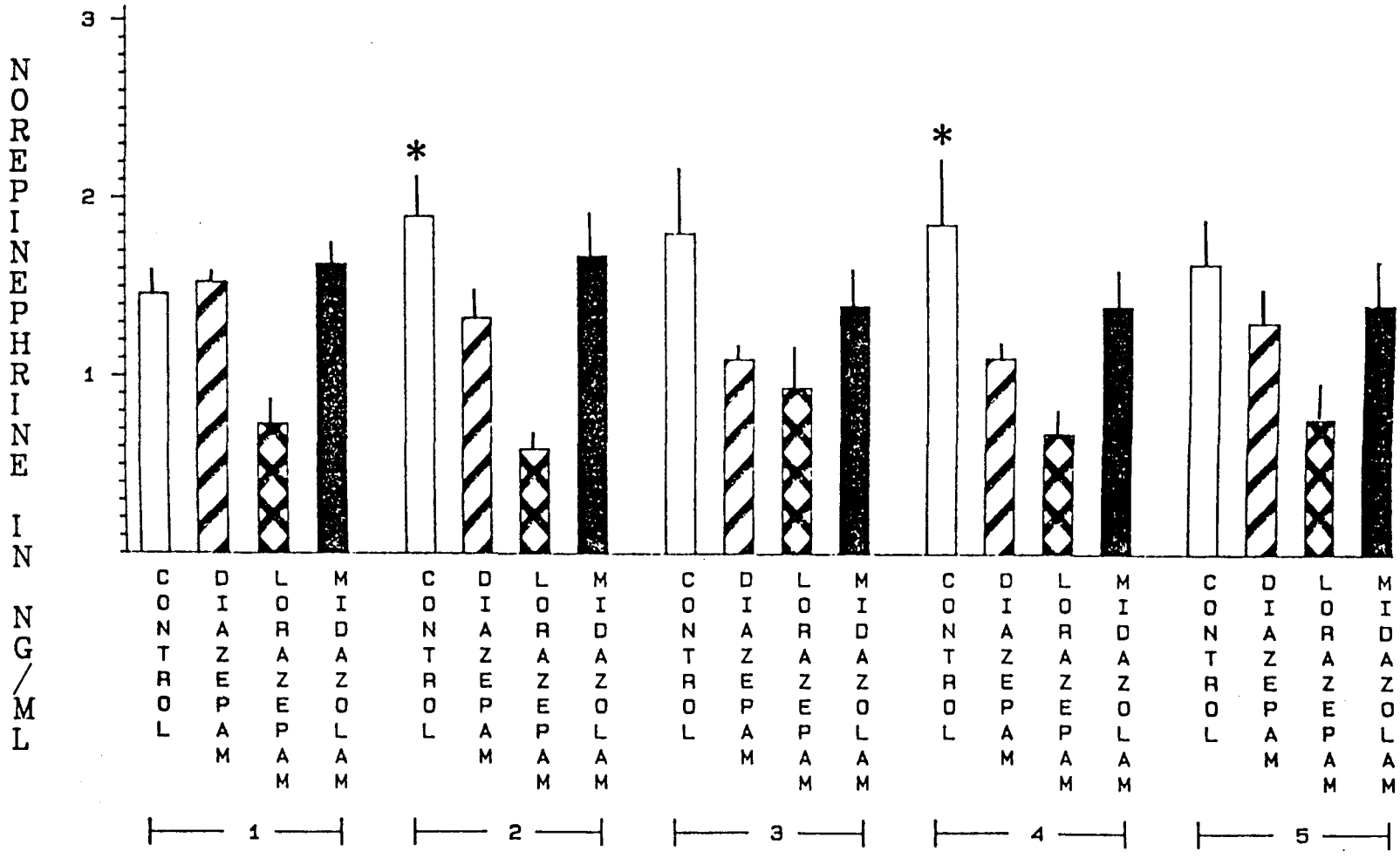

EPINEPHRINE IN NG/ML
3
2
1
CONTROL
DIAZEPAM
LORAZEPAM
MIDAZOLAM
1
2
3
4
5
SAMPLE PERIOD

Norepinephrine. Plasma norepinephrine levels were significantly elevated early in the hypotensive period (Figure 2). However, the increases in plasma norepinephrine were not nearly so dramatic as those for plasma epinephrine. The maximum increase in norepinephrine, occurring 2 minutes after stable hypotension, was only 30% of the prestress control value. During the remainder of the hypotensive period, plasma norepinephrine levels remained close to the 2-minute value, and on recovery they approached prestress control levels.

Benzodiazepine-Treated Animals

Epinephrine. Pretreatment with either diazepam, lorazepam or midazolam attenuated the surge in plasma epinephrine that was seen following the hypotensive stress (Figure 1). The largest effect was seen in the diazepam group at the 2-minute hypotensive preiod. At this time, epinephrine levels which were increased 64% in the control group were only increased 10% in the diazepam group. Furthermore, the maximum increase seen in the diazepam group, occurring 20 minutes into the hypotensive period, was only 16% above the prestress control value. Also, by the recovery period epinephrine levels had returned to the prestress control value.

Figure 2. Comparison of plasma norepinephrine levels (mean plus or minus SEM) before, during and after a 30% decrease in mean arterial blood pressure in dogs receiving saline, diazepam, lorazepam or midazolam prior to the hypotension. Sample periods are: (1) prior to the hypotension (prestress control); (2-4) 2, 10 and 20 minutes after stable hypotension; and (5) 20 minutes after blood pressure returned to prestress control levels. The asterisk indicates a significant difference ($p<.05$) between the indicated value and its corresponding prestress control value.


NOREPINEPHRINE IN NG/ML
3
2
1
*
*
CONTROL
DIAZEPAM
LORAZEPAM
MIDAZOLAM
1
2
3
4
5
SAMPLE PERIOD

Midazolam also attenuated the hypotension-induced rise in plasma epinephrine levels. The maximum attenuation in plasma epinephrine (15% above the control levels) was seen during the 10-minute hypotensive period. At the 2-minute hypotensive period, plasma epinephrine levels were 40% greater than prestress control levels. However, this increase was not statistically significant, presumably due to the large amounts of variability in the data. In general, after the 2-minute period, plasma epinephrine levels fell and remained near prestress control levels throughout the hypotensive and recovery periods.

The results of the lorazepam group are somewhat difficult to interpret since the prestress control epineprhine level in ths group was significantly lower than that in other experimental groups. A review of the events that occurred during the lorazepam experiments did not reveal a specific cause for the lower prestress control value in this group. Lorazepam was given earlier than diazepam or midazolam and its action on catecholamines may have been in progress. However, it does appear that the lorazepam animals responded to the hypotensive stress similarly to the other benzodiazepine-treated animals. The only significant elevation in plasma epinephrine in this group was seen at the 10-minute hypotensive period, and this

value was relatively low. Furthermore, like the other benzodiazepine groups, on recovery of blood pressure plasma epinephrine in the lorazepam group approached prestress control levels.

Norepinephrine. The hypotension-induced rise in plasma norepinephrine levels seen in control animals was attenuated in all benzodiazepine groups. No significant elevations in norepinephrine levels were seen during the hypotensive or recovery periods in any benzodiazepine group (Figure 2). Not only was the hypotension-induced rise in plasma norepinephrine levels attenuated, but a progressive decrease in plasma norepinephrine from prestress control levels occurred in all three benzodiazepine groups. Although the decrease in plasma norepinephrine levels was not statistically significant in any benzodiazepine group, the fall in plasma norepinephrine is of interest since it occurred following administration of all three benzodiazepines. The largest decrease in plasma norepinephrine (28%) from prestress values was seen in the diazepam-treated animals at the 10- and 20-minute hypotensive periods. Lorazepam showed a 19% decrease at the 2-minute hypotensive period, and midazolam showed a 14% decrease at the 10-minute hypotensive period that lasted through the recovery period.

ANOVA

The analysis of variance of plasma epinephrine levels showed both a significant drug and time effect, but the drug*time interaction did not prove to be significant. The significant drug effect was most likely due to a significant difference between the lorazepam animals and other experimental animals, since the other groups did not differ from each other with respect to plasma epinephrine levels.

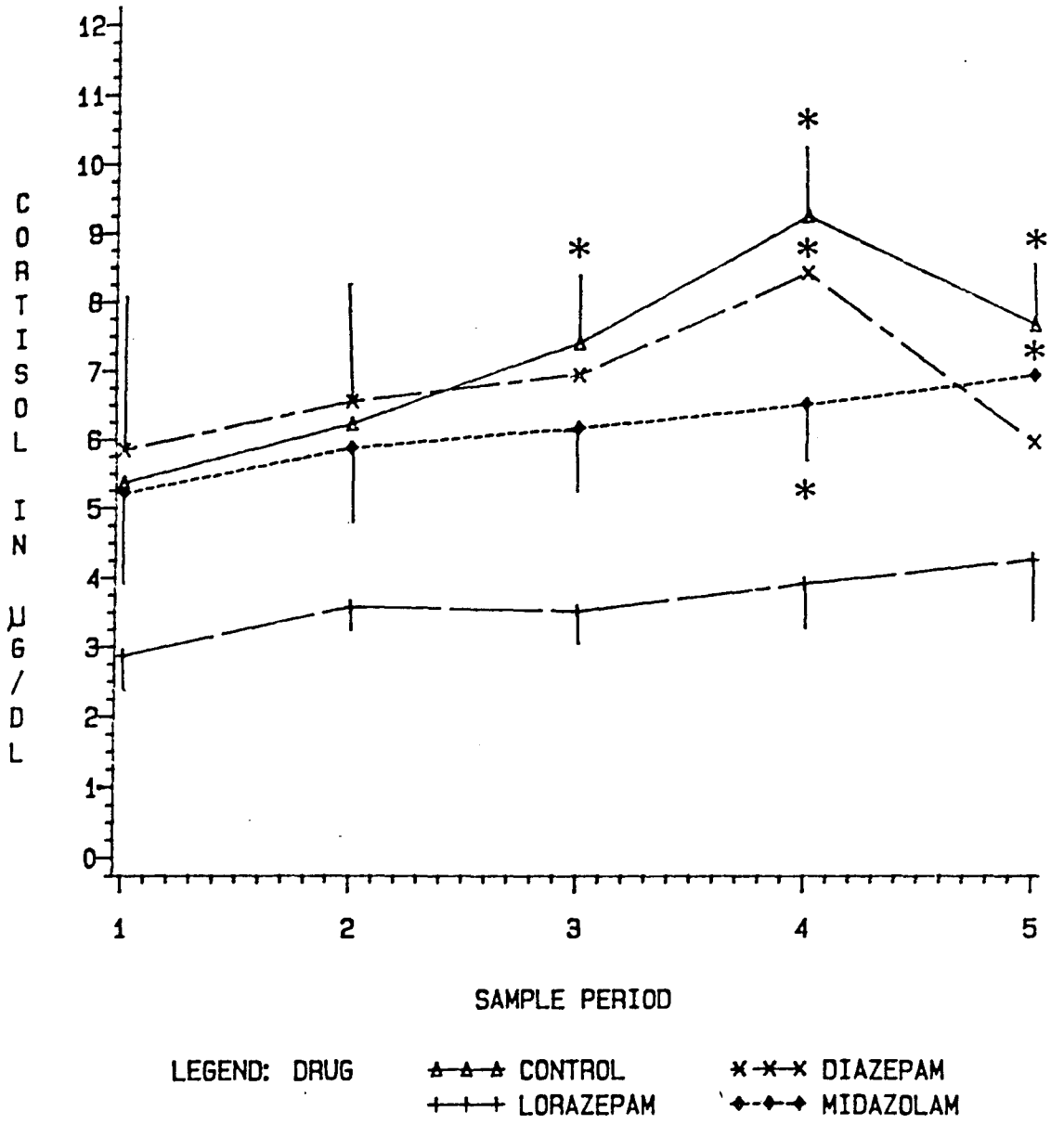
The analysis of variance performed on plasma norepinephrine levels did not show a significant time effect, although a significant drug effect and drug*time interaction were revealed.

Plasma Cortisol Levels

Control Animals

Figure 3 shows that following a hypotensive stress, plasma cortisol levels began increasing and reached statistical significance by the 10 minute hypotensive period. Cortisol levels continued to rise, and by the 20-minute hypotensive period they had increased 73% above prestress control levels. By the recovery period, however, cortisol levels had begun to decrease and approximated the value at the 10-minute hypotensive period.

Figure 3. Comparison of plasma cortisol levels (mean plus or minus SEM) before, during and after a 30% decrease in mean arterial blood pressure in dogs receiving saline, diazepam, lorazepam or midazolam prior to the hypotension. Sample periods are: (1) prior to the hypotension (prestress control); (2-4) 2, 10 and 20 minutes after stable hypotension; and (5) 20 minutes after blood pressure returned to prestress control levels. The asterisk indicates a significant difference ($p<.05$) between the indicated value and its corresponding prestress control value.


CORTISOL IN µG/DL
12
11
10
9
8
7
6
5
4
3
2
1
0
1
2
3
4
5
SAMPLE PERIOD
LEGEND: DRUG
CONTROL
DIAZEPAM
LORAZEPAM
MIDAZOLAM

Benzodiazepine-Treated Animals

The effect of benzodiazepines on plasma cortisol levels (Figure 3) is not as striking as the effect of benzodiazepines on plasma catecholamine levels. Although there was some attenuation of the hypotension-induced surge in plasma cortisol levels, significant increases were still seen in animals pretreated with both diazepam and midazolam. In the diazepam-treated animals, the maximum increase (45%) in plasma cortisol occurred at the 20-minute hypotensive period, but no other significant increases were seen. The midazolam-treated animals showed significant increases in plasma cortisol levels at both the 20-minute hypotensive period and on recovery. The maximum increase, however, was 33%, considerably less than the 73% increase seen in the control animals. The lorazepam-treated animals differed from the other benzodiazepine-treated animals in that no significant elevations in plasma cortisol levels were seen in these animals, although a relatively large (49%) increase was seen on recovery. The lorazepam group also differed from the other experimental groups in that the prestress control plasma cortisol levels of the lorazepam-treated animals were somewhat lower than those of the other experimental groups. No procedural variable was found to account for the lower prestress control levels of the lorazepam group.

As with the catecholamines, lorazepam's action on cortisol may have been in progress.

ANOVA

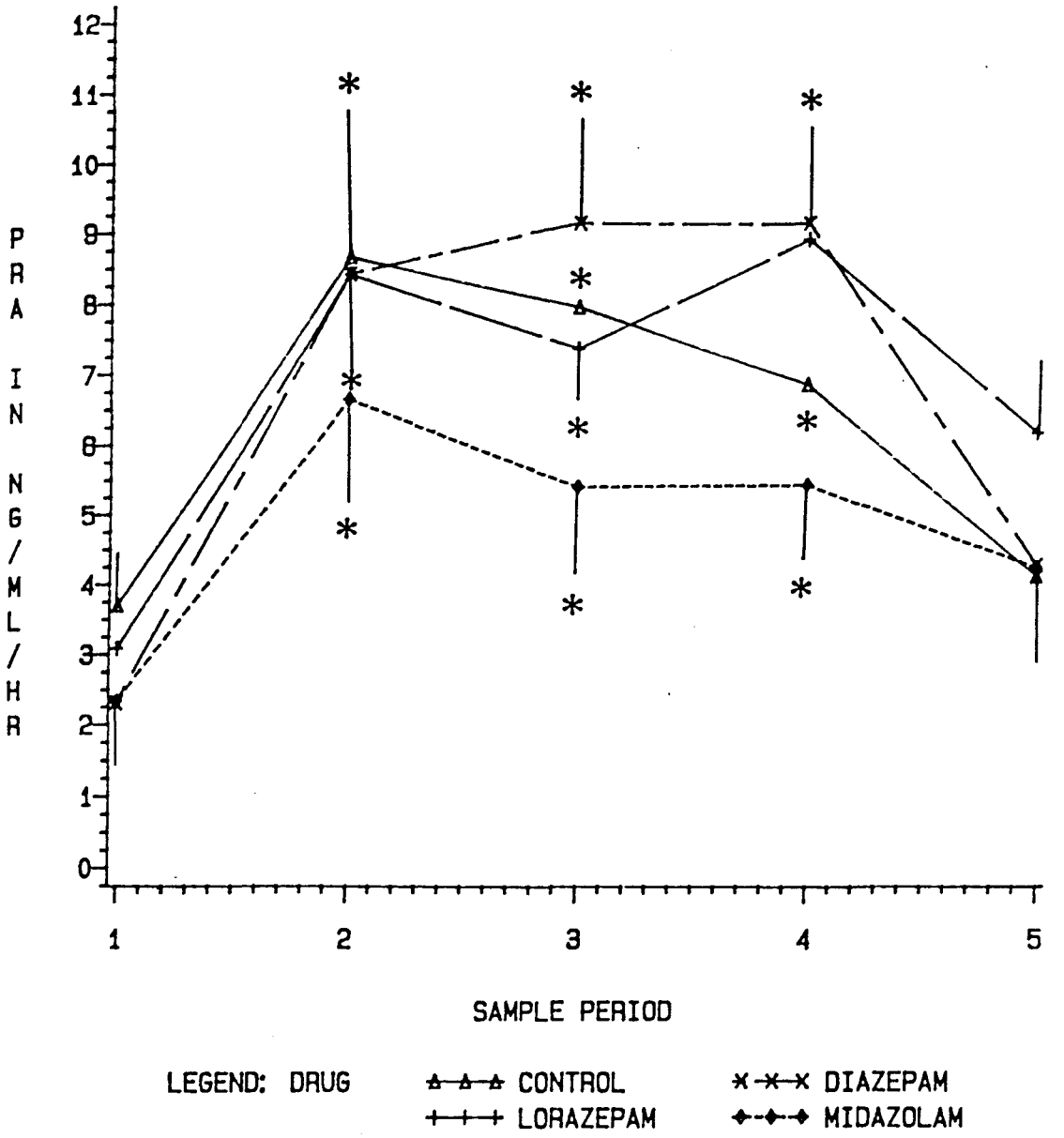
The analysis of variance showed a significant time effect which reflects the elevated plasma cortisol levels seen during the hypotensive and recovery periods in the control, diazepam and midazolam groups. No significant drug effect or drug*time interaction was seen. Thus, the difference between the lorazepam group and the other experimental groups with regard to prestress control plasma cortisol levels, was not statistically significant.

Plasma Renin Activity

Control Animals

The response of plasma renin activity, as shown in Figure 4, was immediate and dramatic. In fact, at the 2-minute hypotensive period, plasma renin activity had increased 134%. Significant elevations in plasma renin activity levels were maintained throughout the hypotensive period; however, on recovery plasma renin activity had fallen almost to prestress control levels.

Figure 4. Comparison of plasma renin activity (mean plus or minus SEM) before, during and after a 30% decrease in mean arterial blood pressure in dogs receiving saline, diazepam, lorazepam or midazolam prior to the hypotension. Sample periods are: (1) prior to the hypotension (prestress control); (2-4) 2, 10 and 20 minutes after stable hypotension; and (5) 20 minutes after blood pressure returned to prestress control levels. The asterisk indicates a significant difference ($p<.05$) between the indicated value and its corresponding prestress control value.


PRA IN NG/ML/HR
12
11
10
9
8
7
6
5
4
3
2
1
0
1
2
3
4
5
SAMPLE PERIOD
LEGEND: DRUG
CONTROL
DIAZEPAM
LORAZEPAM
MIDAZOLAM

Benzodiazepine-Treated Animals

Figure 4 shows that in contrast to the ability of benzodiazepines to attenuate hypotension-induced surges in plasma epinephrine, norepinephrine and cortisol levels, these drugs were relatively ineffective in attenuating the hypotension-induced rise in plasma renin activity. All three benzodiazepine groups showed a response pattern similar to that of the control animals, with significant elevations in plasma renin activity during the hypotensive period that were not maintained on recovery. It is especially interesting to note that plasma renin activity showed greater increases above the prestress control value during the hypotensive stress in all benzodiazepine groups than in the control group. The maximum increase in the control group was 134%. Of the benzodiazepine groups, diazepam showed a maximum increase of 298%, lorazepam showed a maximum increase of 191% and midazolam showed a maximum increase of 185%.

ANOVA

The analysis of variance of plasma renin activity showed a significant time effect. However, no significant drug effect or drug*time interactions were found.

Experiment II

Hemodynamic Measurements

Control Animals

In this experiment, the effects of midazolam administered 1-1/2 hours before the hypotensive stress were investigated. Results from the control group, which were reported in Experiment I, are redrawn on the figures relating to this experiment in order to facilitate comparisons between control and midazolam-treated animals.

Midazolam-Treated Animals

The hemodynamic profile of animals injected with midazolam 1-1/2 hours before nitroprusside-induced hypotension is shown in Table 5. Blood pressure was maintained at approximately 30% of prestress control periods during the nitroprusside infusion, and a moderate, but statistically insignificant increase above the control value, was seen on recovery. Heart rate was well maintained during the hypotensive period, but it was significantly decreased on recovery. Cardiac index fell during the hypotensive period, and

Table 5. Hemodynamic profile of dogs injected with midazolam 1-1/2 hours prior to a nitroprusside-induced 30% decrease in mean arterial blood pressure. Abbreviations used are: MAP, mean arterial blood pressure; HR, heart rate; CI, cardiac index; RAP, right atrial pressure; SVRI, systemic vascular resistance index; Wedge, pulmonary capillary wedge pressure; and LVSWI, left ventricular stroke work index. Values represent means plus or minus SEM. The asterisk indicates a significant difference between the indicated value and its corresponding prestress control value.

TABLE 5

MIDAZOLAM GIVEN 1-1/2 HOURS PRESTRESS: HEMODYNAMICS

	Prestress Control	Hypotension			20 Min Post-Recovery
		2 Min	10 Min	20 Min	
MAP (mm Hg)	85±3	56±2*	58±2*	59±2*	91±3
HR (min^{-1})	124±6	122±4	119±4	119±4	112±5*
CI ($L \cdot min^{-1} \cdot M^{-2}$)	4.9±0.5	3.9±0.3*	4.0±0.3*	4.1±0.2*	4.6±0.1
RAP (mm Hg)	4.3±0.9	2.9±0.7	3.3±0.7	2.9±0.8	5.3±0.9
SVRI ($dyne \cdot sec \cdot cm^{-5} \cdot M2$)	1383±151	1096±72*	1129±97*	1105±73*	1505±93
Wedge (mm Hg)	10.5±1.9	8.7±2.1*	8.0±2.1*	8.2±2.1*	10.7±1.7
LVSWI ($gm \cdot M \cdot M^{-2}$)	40±4	20±2*	23±1*	24±2*	44±3

rose to approximately the prestress control level. Contrary to the relatively stable systemic vascular resistance index seen in the control group, this variable was significantly decreased in the hypotensive period in the midazolam group.

Changes in stroke work index and right atrial pressure during the hypotensive and recovery periods in the midazolam group paralleled changes in the control group, which were shown in Table 1. That is, significant decreases in stroke work index were seen during the hypotensive period, and no statistical differences in right atrial pressure were reported during the hypotensive or recovery periods.

A comparison of the hemodynamic profile of midazolam in the first experiment (midazolam I, Table 4) with the hemodynamic profile of midazolam in this experiment (midazolam II, Table 5) showed some interesting differences. Heart rate and right atrial pressure were better maintained in the midazolam II group, while systemic vascular resistance index and wedge pressure were better maintained in the midazolam I group. Other hemodynamic variables such as cardiac index and stroke work index, however, showed similar changes in both midazolam groups.

ANOVA

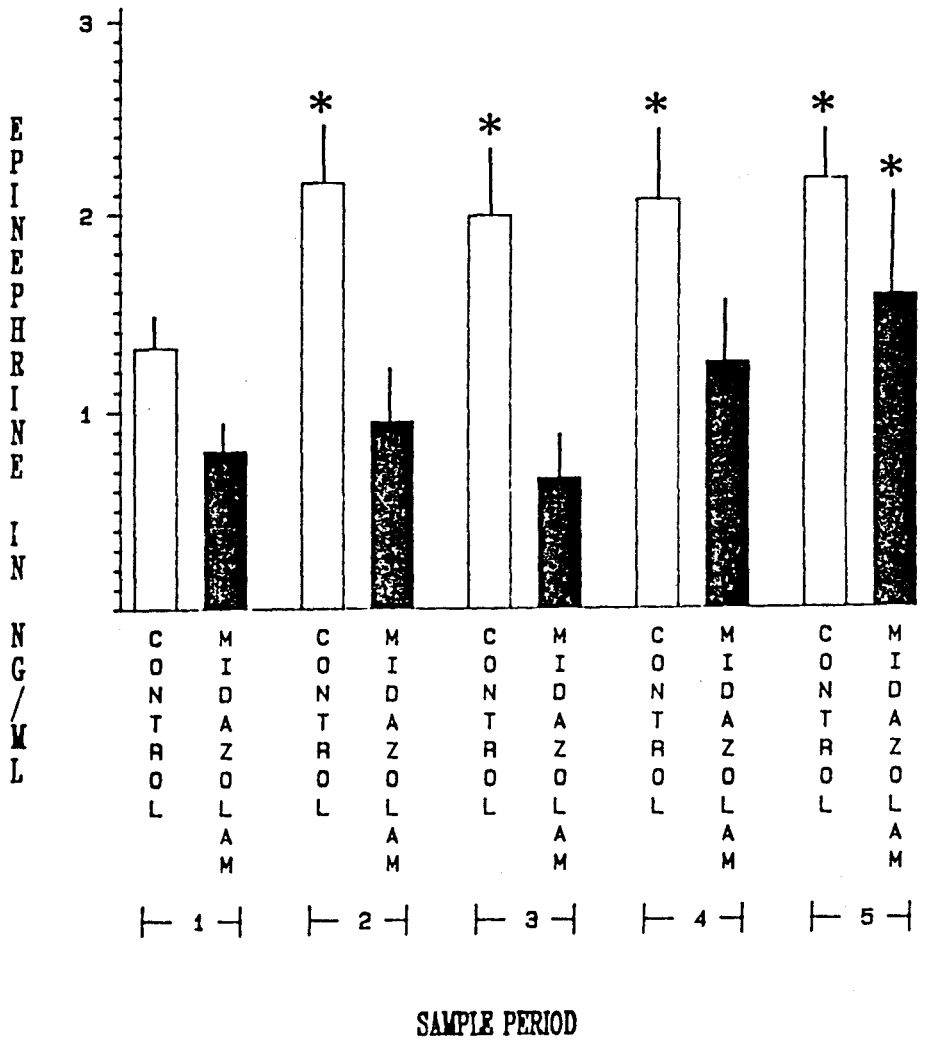
An analysis of variance of control and midazolam II groups showed a significant time effect for all hemodynamic variables. A significant drug effect was found for cardiac index, systemic vascular resistance index and wedge pressure. The only drug*time effect was seen with respect to wedge pressure, and is most likely due to the significant decreases seen during the hypotensive and recovery periods in the midazolam II group, but not in the control group.

Plasma Catecholamine Levels

Midazolam-Treated Animals

Epinephrine. Figure 5 shows plasma epinephrine levels in the control midazolam II groups. During the hypotensive period, plasma epinephrine levels in the midazolam II group remained near the prestress level. At the 20-minute hypotensive period; however, a statistically nonsignificant 55% increase was observed. As in the control group, plasma epinephrine levels increased in the midazolam II group to reach statistical significance in the recovery period. In fact, the 98% increase from prestress control levels in the recovery period seen in the midazolam II group even exceeded

Figure 5. Comparison of plasma epinephrine levels (mean plus or minus SEM) before, during and after a 30% decrease in mean arterial blood pressure in dogs receiving midazolam 1-1/2 hours prior to the hypotension. Control values from experiment I are redrawn on this figure. Sample periods are: (1) prior to the hypotension (prestress control); (2-4) 2, 10 and 20 minutes after stable hypotension; and (5) 20 minutes after blood pressure returned to prestress control levels. The asterisk indicates a significant difference ($p<.05$) between the indicated value and its corresponding prestress control value.


EPINEPHRINE IN NG/ML
3
2
1
*
*
*
*
*
CONTROL
MIDAZOLAM
1
2
3
4
5
SAMPLE PERIOD

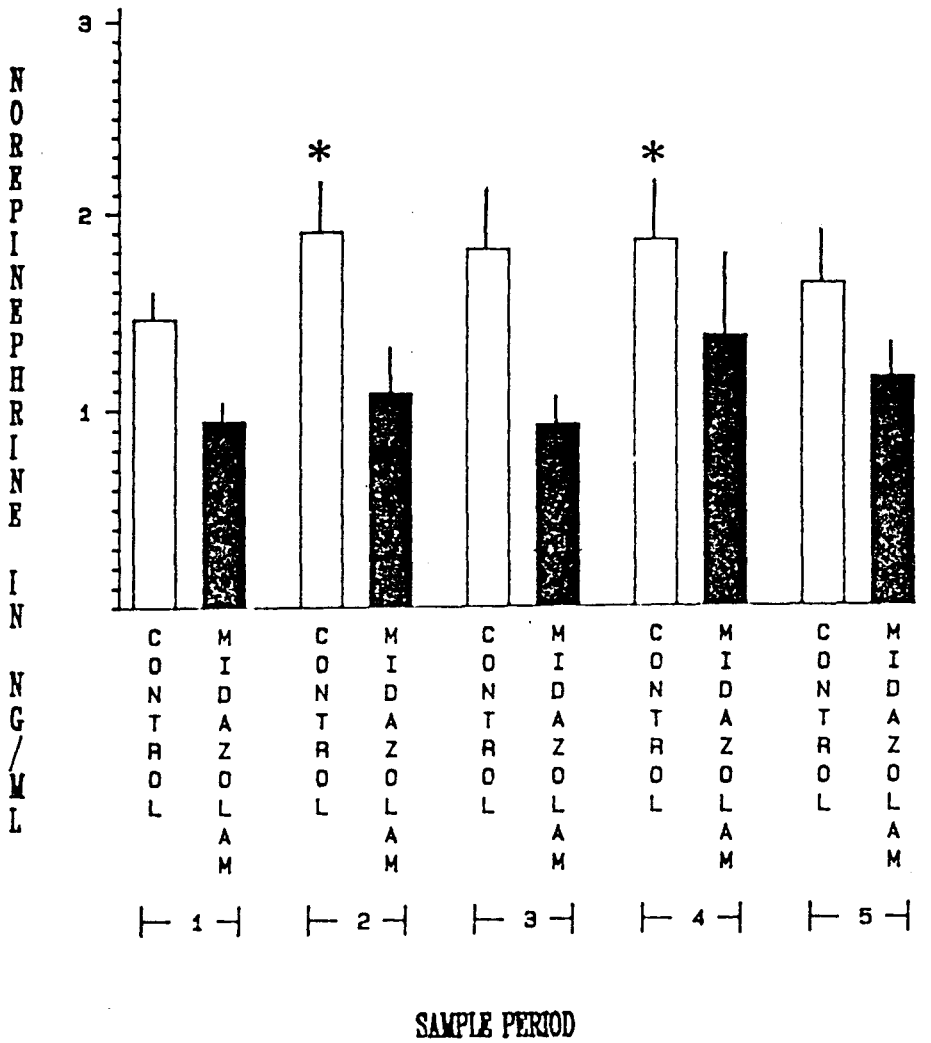
the 64% increase seen in the control group, although the absolute values in the midazolam II group were somewhat less than those of the control group.

Plasma epinephrine levels in the midazolam II group (Figure 5) changed in a manner similar to that of the midazolam I group (Figure 1) during the hypotensive period, since neither group showed any significant elevations above the prestress control period during this time. However, the two midazolam groups differed with respect to plasma epinephrine levels at the recovery period. Plasma epinephrine levels peaked at this time period in the midazolam II group, but they approached prestress control levels in the midazolam I group.

Norepinephrine. Plasma norepinephrine levels during the hypotensive and recovery periods are shown in Figure 6 In contrast to the significant elevations in plasma norepinephrine seen during the hypotensive period in the control group, plasma norepinephrine levels in the midazolam II group remained relatively stable, with no significant increase seen during either the hypotensive or recovery periods.

In both midazolam study groups the surge in plasma norepinephrine levels following the hypotensive stress was attenuated by pretreatment with midazolam given either 4

Figure 6. Comparison of plasma norepinephrine levels (mean plus or minus SEM) before, during and after a 30% decrease in mean arterial blood pressure in dogs receiving midazolam 1-1/2 hours prior to the hypotension. Control values from experiment I are redrawn on this figure. Sample periods are: (1) prior to the hypotension (prestress control); (2-4) 2, 10 and 20 minutes after stable hypotension; and (5) 20 minutes after blood pressure returned to prestress control levels. The asterisk indicates a significant difference ($p<.05$) between the indicated value and its corresponding prestress control value.


NOREPINEPHRINE IN NG/ML
3
2
1
*
*
CONTROL
MIDAZOLAM
1
2
3
4
5
SAMPLE PERIOD

minutes or 1-1/2 hours prior to the stress. The only difference between the midazolam I (Figure 2) and midazolam II (Figure 6) groups was that plasma norepinephrine levels were somewhat lower in the midazolam II group, and this difference was statistically significant at the prestress control and 10-minute hypotensive periods.

ANOVA

When the plasma catecholamine levels in the control group were compared with those of the midazolam II group using the analysis of variance procedure, significant time and drug effects were found with respect to plasma epinephrine levels, and a significant drug effect was found with respect to plasma norepinephrine levels. The significant drug effect found for both catecholamines reflects the lower values of the midazolam II group.

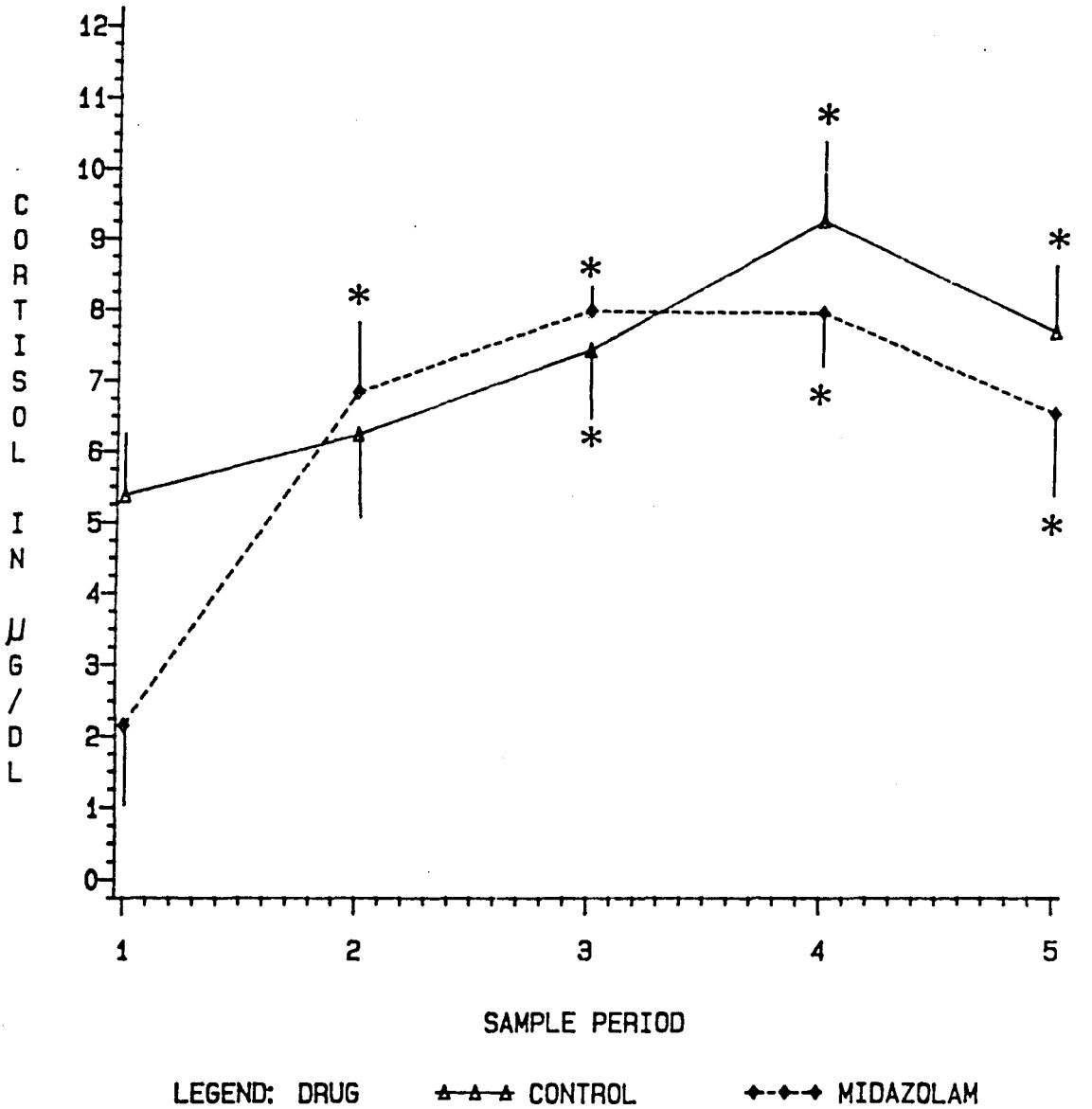
Plasma Cortisol Levels

Midazolam-Treated Animals

Plasma cortisol levels increased during the hypotensive and recovery periods in the control dogs and animals given midazolam 1-1/2 hours prestress (Figure 7). At the 2-minute hypotensive period, plasma cortisol levels had increased significantly in the midazolam, but not control groups. Plasma cortisol levels in the midazolam II group then increased 227% above prestress control levels at the 10-minute hypotensive period, were maintained at the 20-minute hypotensive period, but fell on recovery. The response of the midazolam II group thus was considerably greater than that of the control group, since the maximum increase in the control group (73%) was not seen until the 20-minute hypotensive period. The greater percentage increase in the midazolam II group, however, is related to the prestress control value which was statistically lower than that of the control group.

A comparison of the midazolam I (Figure 3) and midazolam II (Figure 7) groups showed significant elevations in plasma cortisol levels at the 20-minute hypotensive and recovery periods in both groups; however, the response of these groups differed during the early stages of

Figure 7. Comparison of plasma cortisol levels (mean plus or minus SEM) before, during and after a 30% decrease in mean arterial blood pressure in dogs receiving midazolam 1-1/2 hours prior to the hypotension. Control values from experiment I are redrawn on this figure. Sample periods are: (1) prior to the hypotension (prestress control); (2-4) 2, 10 and 20 minutes after stable hypotension; and (5) 20 minutes after blood pressure returned to prestress control levels. The asterisk indicates a significant difference ($p<.05$) between the indicated value and its corresponding prestress control value.


CORTISOL IN µG/DL
12
11
10
9
8
7
6
5
4
3
2
1
0
1
2
3
4
5
SAMPLE PERIOD
LEGEND: DRUG
CONTROL
MIDAZOLAM

hypotension. That is, at the 2- and 10-minute hypotensive periods, the significant elevations in plasma cortisol levels seen in the midazolam II group were not seen in the midazolam I group. No significant differences, however, were found at any time period when plasma cortisol levels in the midazolam I group were compared with those of the midazolam II group.

ANOVA

An analysis of variance of the control and midazolam II plasma cortisol levels showed a significant time effect and drug*time interaction. The lack of a significant drug effect indicates that there were no statistically significant differences between these groups. The significant drug*time interaction is somewhat puzzling, since in both groups plasma cortisol increased during hypotension and then fell somewhat by the recovery period; however, the significant effect is most likely related to the lower prestress control values in the midazolam group.

Experiment III

Hemodynamic Measurements

Control Animals

The hemodynamic profile of control animals in response to thoracotomies is shown in Table 6. Two minutes after the retractor was placed between the ribs, blood pressure rose slightly, then fell. By 10 minutes of rib spread blood pressure was significantly lower than the prestress control level. Heart rate fell slightly and significantly 2 minutes after the thoracotomy and, like blood pressure, continued to fall throughout the experiment. This downward trend was also seen with regard to cardiac index and stroke work index, with these hemodynamic parameters reaching statistical significance at 5 and 10 minutes, respectively.

Right atrial pressure, systemic vascular resistance index and wedge pressure, the only hemodynamic variables to show any upward movement, did not reach statistical significance. Systemic vascular resistance index was elevated during the surgical stress, but this increase did not reach statistical significance.

Table 6. Hemodynamic profile of control (saline-treated) dogs in response to surgical stress. Timing began after the retractor was placed between the 5th and 6th ribs. Abbreviations used are: MAP, mean arterial blood pressure; HR, heart rate; CI, cardiac index; RAP, right atrial pressure; SVRI, systemic vascular resistance index; Wedge, pulmonary capillary wedge pressure; and LVSWI, left ventricular stroke work index. Values represent means plus or minus SEM. The asterisk indicates a significant difference between the indicated value and its corresponding prestress control value.

TABLE 6

HEMODYNAMIC PROFILE OF CONTROL DOGS: SURGICAL STRESS

	Prestress Control	Rib Spread				20 Min Post-Rib Spread
		2 Min	5 Min	10 Min	20 Min	
MAP (mm Hg)	85±7	89±5	83±6	79±4*	75±4*	71±4*
HR (min^{-1})	112±5	107±2*	107±2*	106±2*	104±2*	104±3*
CI ($L \cdot min^{-1} \cdot M^{-2}$)	3.7±0.3	3.4±0.3	3.3±0.3*	3.2±0.3*	3.1±0.4*	2.9±0.4*
RAP (mm Hg)	3.8±0.4	3.4±0.7	3.5±0.8	4.5±0.4	4.3±0.3	4.3±0.2
SVRI ($dyne \cdot sec \cdot cm^{-5} \cdot M^2$)	1747±156	2061±193	2023±204	1943±205	2004±292	1962±237
Wedge (mm Hg)	7.7±0.8	8.5±0.4	8.3±0.4	8.5±0.5	8.0±0.4	8.2±0.8
LVSWI ($gm \cdot M \cdot M^{-2}$)	37±6	34±3	31±4	29±3*	28±4*	24±3*

Midazolam-Treated Animals

The hemodynamic profile of the midazolam-treated animals, shown in Table 7, is quite similar to that of the control animals. The midazolam group, like the control group, showed significant decreases in mean arterial blood pressure, heart rate, cardiac index and stroke work index; and nonsignificant increases in right atrial pressure and systemic vascular resistance index. In fact, the only difference between these two groups was in wedge pressure which increased slightly, but significantly, above the prestress control level only in the midazolam group.

ANOVA

The analysis of variance showed a significant time effect for all hemodynamic variables except systemic vascular resistance index and right atrial pressure. No significant drug effect or drug*time interaction were found.

Table 7. Hemodynamic profile of midazolam-treated dogs in response to surgical stress. Timing began after the retractor was placed between the 5th and 6th ribs. Abbreviations used are: MAP, mean arterial blood pressure; HR, heart rate; CI, cardiac index; RAP, right atrial pressure; SVRI, systemic vascular resistance index; Wedge, pulmonary capillary wedge pressure; and LVSWI, left ventricular stroke work index. Values represent means plus or minus SEM. The asterisk indicates a significant difference between the indicated value and its corresponding prestress control value.

TABLE 7

HEMODYNAMIC PROFILE OF MIDAZOLAM DOGS: SURGICAL STRESS

	Prestress Control	Rib Spread				20 Min Post-Rib Spread
		2 Min	5 Min	10 Min	20 Min	
MAP (mm Hg)	89±5	84±4	79±3*	76±3*	75±3*	69±5*
HR (min^{-1})	122±4	118±5	118±6	116±5*	115±5*	112±6*
CI ($L \cdot min^{-1} \cdot M^{-2}$)	3.2±0.3	2.8±0.4	2.6±0.4*	2.8±0.3	2.7±0.4*	2.3±0.4*
RAP (mm Hg)	4.0±0.5	4.3±0.6	4.2±0.5	3.9±0.6	4.3±0.6	4.3±0.4
SVRI ($dyne \cdot sec \cdot cm^{-5} \cdot M^2$)	2178±221	2368±254	2503±290	2161±198	2189±206	2681±680
Wedge (mm Hg)	7.3±1.2	8.3±1.2*	8.3±1.4*	8.3±1.3*	8.5±1.2*	8.0±1.1
LVSWI ($gm \cdot M \cdot M^{-2}$)	30±4	25±3*	22±4*	23±3*	22±4*	18±4*

Plasma Catecholamine Levels

Control Animals

Epinephrine. Figure 8 shows the plasma epinephrine levels following placement of the retractor and after its removal. No statistically significant elevations in plasma epinephrine levels were seen in the control animals. This is somewhat surprising, considering that 5 minutes after rib spread plasma epinephrine levels had increased 119%, and 20 minutes after removal of the retractor the increase was 141%. However, on a closer examination of the data, a very high degree of variability is revealed. In fact, the coefficient of variation (the ratio of the standard deviation divided by the mean and multiplied by 100) was 102% 5 minutes after rib spread and 75% 20 minutes after removal of the retractor.

Norepinephrine. Plasma norepinephrine levels, like plasma epinephrine levels, did not increase significantly in response to surgical stress in the control animals (Figure 9). However, the changes in plasma norepinephrine levels in this group were not as great as those in plasma epinephrine, since the maximum increase during rib spread was only 14% and the increase seen after removal of the retractor was 47%. This data was also somewhat variable since the coeffi-

Figure 8. The effect of pretreatment with midazolam on plasma epinephrine levels following surgical stress. Sample periods are: (1) prior to skin incision (prestress control); (2-5) 2, 5, 10 and 20 minutes after placement of the retractor; and (6) 20 minutes after removal of the retractor.

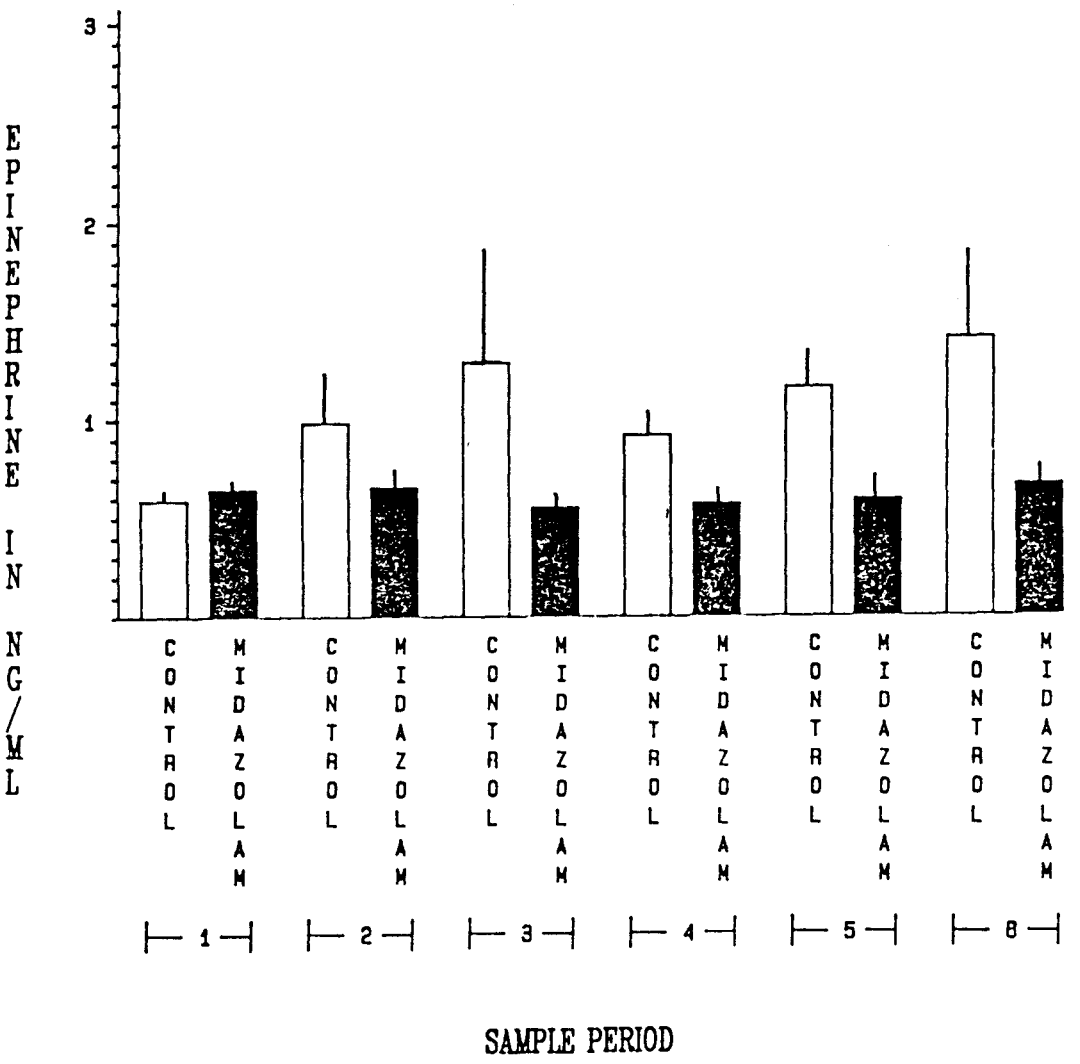
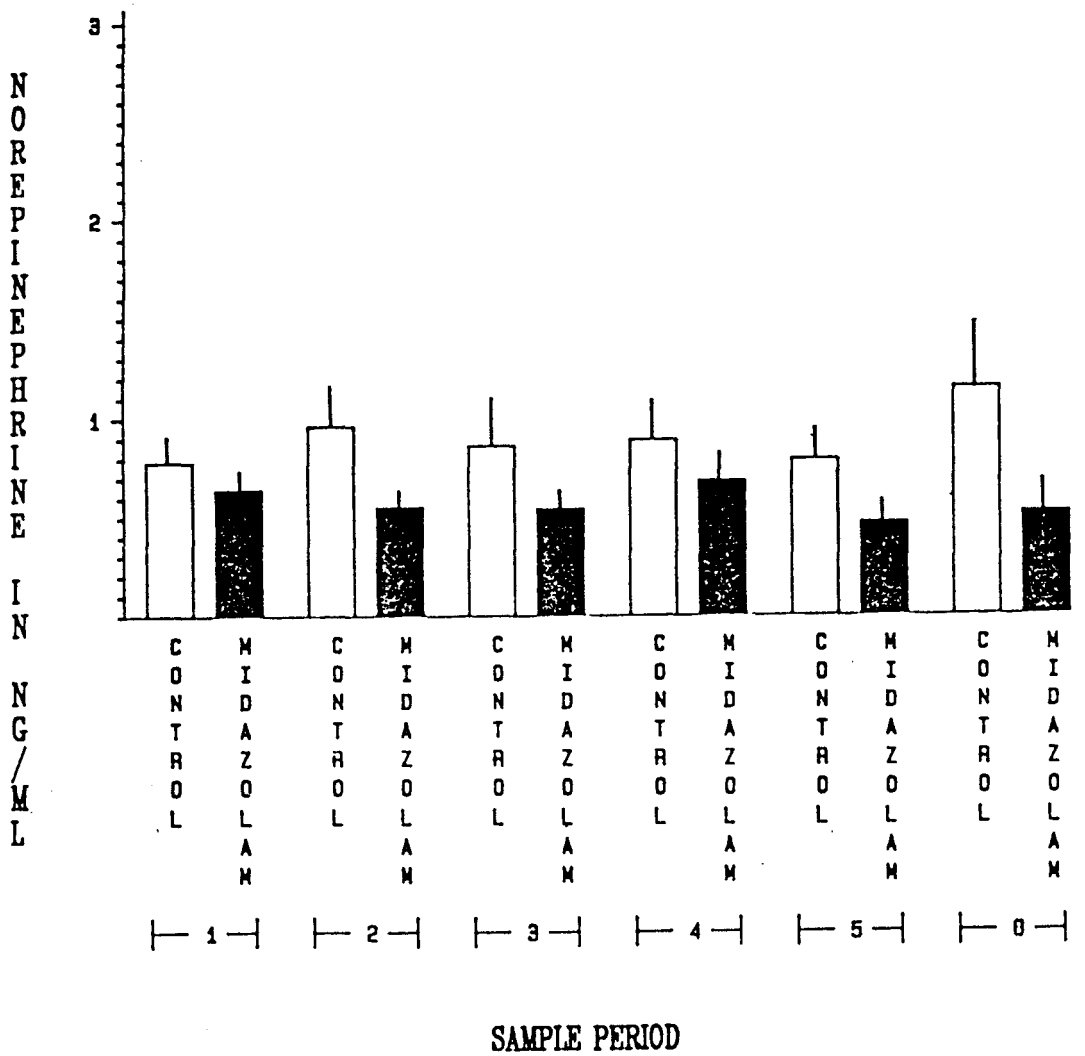

EPINEPHRINE IN NG/ML
3
2
1
CONTROL
MIDAZOLAM
1
2
3
4
5
6
SAMPLE PERIOD

Figure 9. The effect of pretreatment with midazolam on plasma norepinephrine levels following surgical stress. Sample periods are: (1) prior to skin incision (prestress control); (2-5) 2, 5, 10 and 20 minutes after placement of the retractor; and (6) 20 minutes after removal of the retractor.


NOREPINEPHRINE IN NG/ML
3
2
1
CONTROL
MIDAZOLAM
1
2
3
4
5
0
SAMPLE PERIOD

cient of variation was over 50% both during rib spread and following removal of the retractor.

Midazolam-Treated Animals

Epinephrine. As shown in Figure 8, no significant elevations in plasma epinephrine levels were seen in response to the thoracotomy procedure in the midazolam-treated animals. However, unlike the control animals, the elevations in plasma epinephrine in the midazolam group were slight, reaching a maximum increase of 3% 20 minutes after removal of the retractor, and even decreasing slightly 5, 10 and 20 minutes after rib spread. Furthermore, the amount of variablility seen in these data was considerably less than that seen in the control group, since the maximum coefficient of variation, which occurred 2 minutes after rib spread, was only 38% in the midazolam group.

Norepinephrine. Figure 9 shows that no significant changes in plasma norepinephrine were seen in this experiment. However, it is interesting to note that at all time periods except 10 minutes after rib spread, plasma norepinephrine levels were decreased slightly from the prestress control value. For the most part, these data did not show much variability, although 20 minutes after rib spread and

removal of the retractor, the coefficient of variation was above 50%. It should be noted that the baseline epinephrine and norepinephrine levels in the surgically stressed dogs were lower than baseline levels in hypotensively stressed dogs (cf., Figures 5 and 6). The larger 15-18 kg dogs used in the surgery experiments were found to have lower resting catecholamine levels compared with the 12-14 kg dogs used earlier in the study.

ANOVA

When plasma catecholamine levels from the control and midazolam groups were subjected to an analysis of variance, only the drug effect for epinephrine was significant. This suggests that although plasma epinephrine did not show a statistically significant increase over the prestress control value in the control group, this group did respond differently from the midazolam-treated animals. Further analysis revealed that the control and midazolam groups differed significantly from each other with regard to plasma epinephrine levels 10 and 20 minutes after rib spread.

Plasma Cortisol Levels

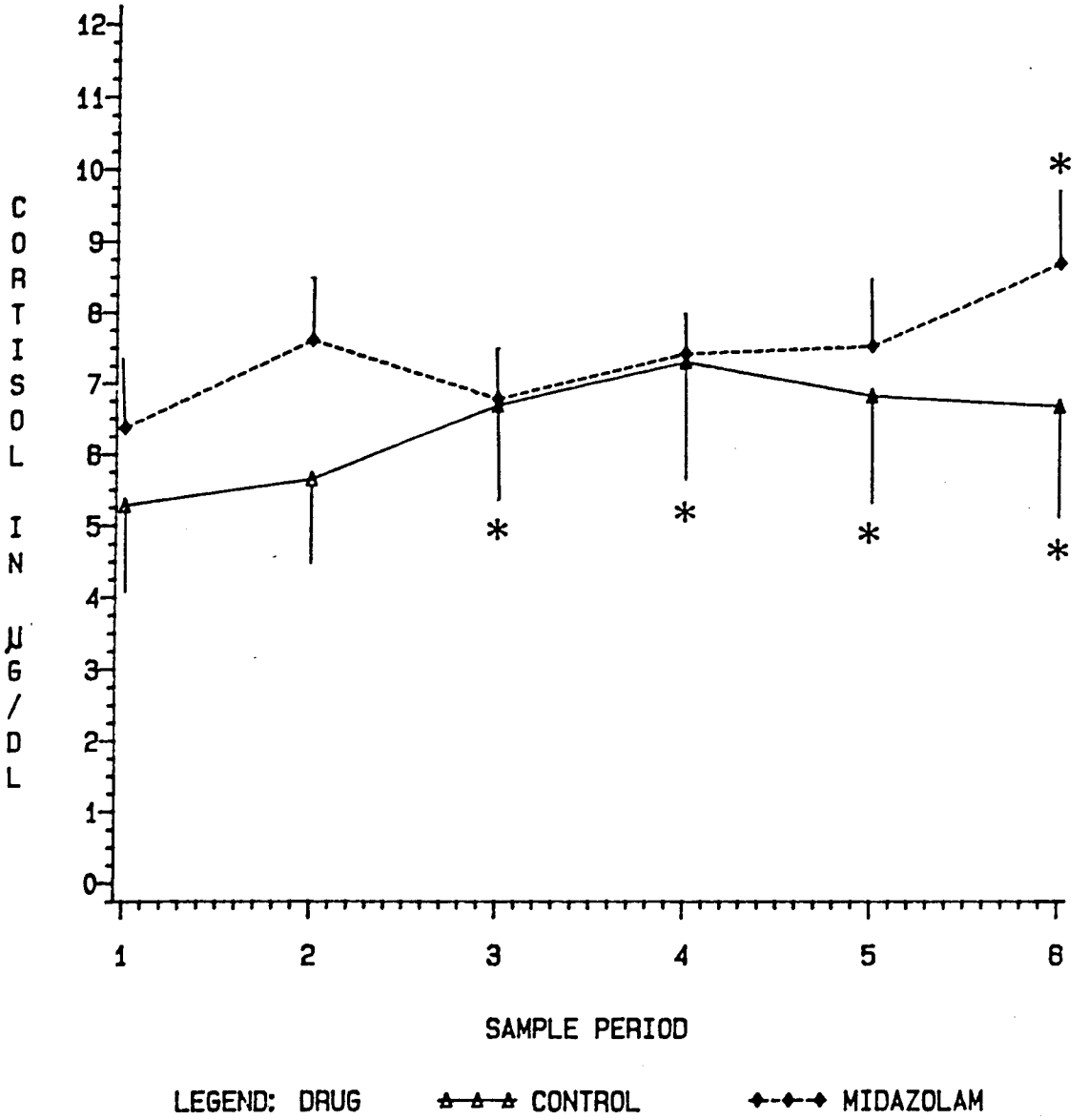
Control Animals

Figure 10 shows that surgical stress resulted in significant increases in plasma cortisol levels in control animals. Plasma cortisol levels were significantly increased from 5 minutes after rib spread until the end of the experiment, with a maximum increase of 38% occurring 10 minutes after rib spread.

Midazolam-Treated Animals

Midazolam was as effective in attenuating the surgery-induced increase in plasma cortisol levels as it was in attenuating the hypotension-induced increase in cortisol. Figure 10 shows that no statistically significant rises in plasma cortisol levels in the midazolam group occurred while the retractor was in place, although the levels did gradually increase during rib spread. Twenty minutes after the retractor was removed, however, cortisol levels increased significantly to 37% above the prestress control value.

Figure 10. The effect of pretreatment with midazolam on plasma cortisol levels following surgical stress. Sample periods are: (1) prior to skin incision (prestresss control); (2-5) 2, 5, 10 and 20 minutes after placement of the retractor; and (6) 20 minutes after removal of the retractor. The asterisk indicates a significant difference ($p<.05$) between the indicated value and its corresponding prestress control value.


CORTISOL IN µG/DL
12
11
10
9
8
7
6
5
4
3
2
1
0
1
2
3
4
5
6
SAMPLE PERIOD
LEGEND: DRUG
CONTROL
MIDAZOLAM

ANOVA

A comparison of plasma cortisol levels in the control and midazolam groups using the analysis of variance showed significant time effect. However, no significant drug effect or drug*time interaction was found.

Plasma Renin Activity

Control Animals

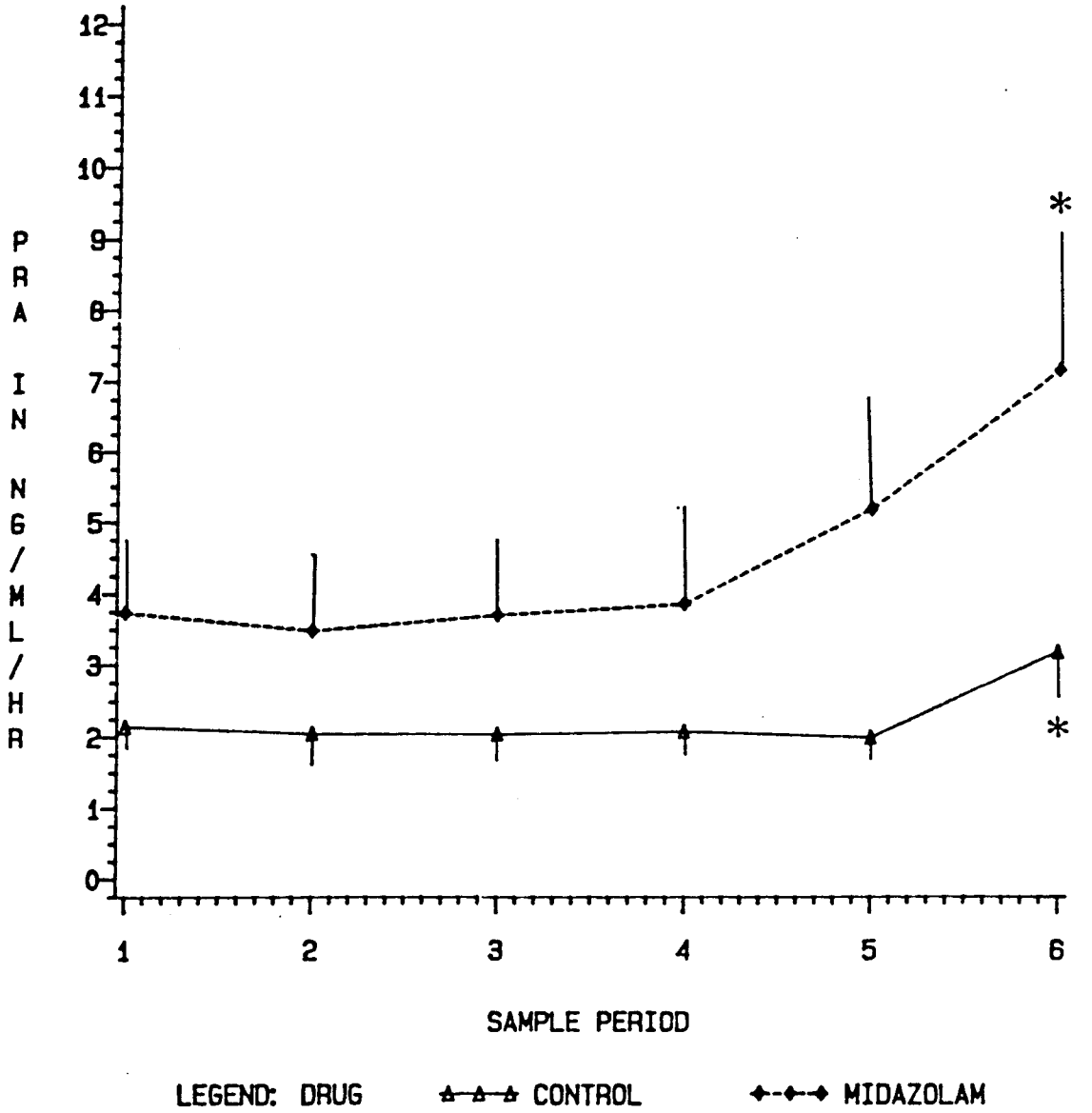
The changes in plasma renin activity in control animals in response to surgical stress are shown in Figure 11. In contrast to the large elevation in plasma renin activity seen following a hypotensive stress (Figure 4), plasma renin activity remained quite stable during the 20 minutes that the retractor was in place between the ribs. However, 20 minutes after the retractor was removed, plasma renin activity increased 49% above prestress control values. This increase, while statistically significant, is quite moderate compared with the 134% increase in control animals during the hypotensive stress.

Midazolam-Treated Animals

The response of plasma renin activity in animals pretreated with midazolam is shown in Figure 11. In these animals, plasma renin activity showed an upward trend from 5 minutes after rib spread until the end of the experiment. Like the control group, no significant changes in plasma renin activity were seen during the 20 minutes that the retractor was in place; however, 20 minutes after removal of the retractor, plasma renin activity rose significantly.

Although the plasma renin activity response in control and midazolam-treated animals was statistically similar, the magnitude of the response of the animals pretreated with midazolam was somewhat greater. For example, 20 minutes after rib spread, plasma renin activity had increased 39% over the prestress control value in the midazolam-treated animals and decreased 7% in the control animals. Furthermore, 20 minutes after removal of the retractor, a 93% increase over prestress control values was seen in the midazolam-treated animals, while only a 49% increase was seen in the control animals. Despite the appearance of large differences between the control and midazolam groups, no statistically significant difference was found between these groups at any time period.

Figure 11. The effect of pretreatment with midazolam on plasma renin activity following surgical stress. Sample periods are: (1) prior to skin incision (prestress control); (2-5) 2, 5, 10 and 20 minutes after placement of the retractor; and (6) 20 minutes after removal of the retractor. The asterisk indicates a significant difference ($p<.05$) between the indicated value and its corresponding prestress control value.


PRA IN NG/ML/HR
12
11
10
9
8
7
6
5
4
3
2
1
0
*
*
1
2
3
4
5
6
SAMPLE PERIOD
LEGEND: DRUG
CONTROL
MIDAZOLAM

ANOVA

A comparison of plasma renin activity in the control and midazolam groups using an analysis of variance showed a significant time effect and drug*time interaction and an insignificant drug effect.

Heart Rate and Blood Pressure Prior to Rib Spread

Control Animals

Each minute from the time of skin incision until placement of the retractor, heart rate and blood pressure measurements were taken. Figures 12 and 13 show mean arterial blood pressure and heart rate in control animals in response to skin incision, blunt dissection of muscle tissue and perforation of the pleural cavity that occurred during the 5 minutes prior to placement of the retractor. Figure 12 shows that blood pressure increased above the preincision control level following skin incision. This increase in blood pressure reached statistical significance 4 minutes after skin incision, dropped slightly five minutes after skin incision, but maintained statistical significance. Heart rate, on the other hand, showed a very slight downward trend, only reaching statistical significance 5 minutes after skin incision.

Figure 12. The effect of midazolam on mean arterial blood pressure prior to placement of the retractor between the 5th and 6th ribs. Timing began immediately before skin incision and continued for five minutes. The asterisk indicates a significant difference ($p<.05$) between the indicated value and its corresponding preincision control value.

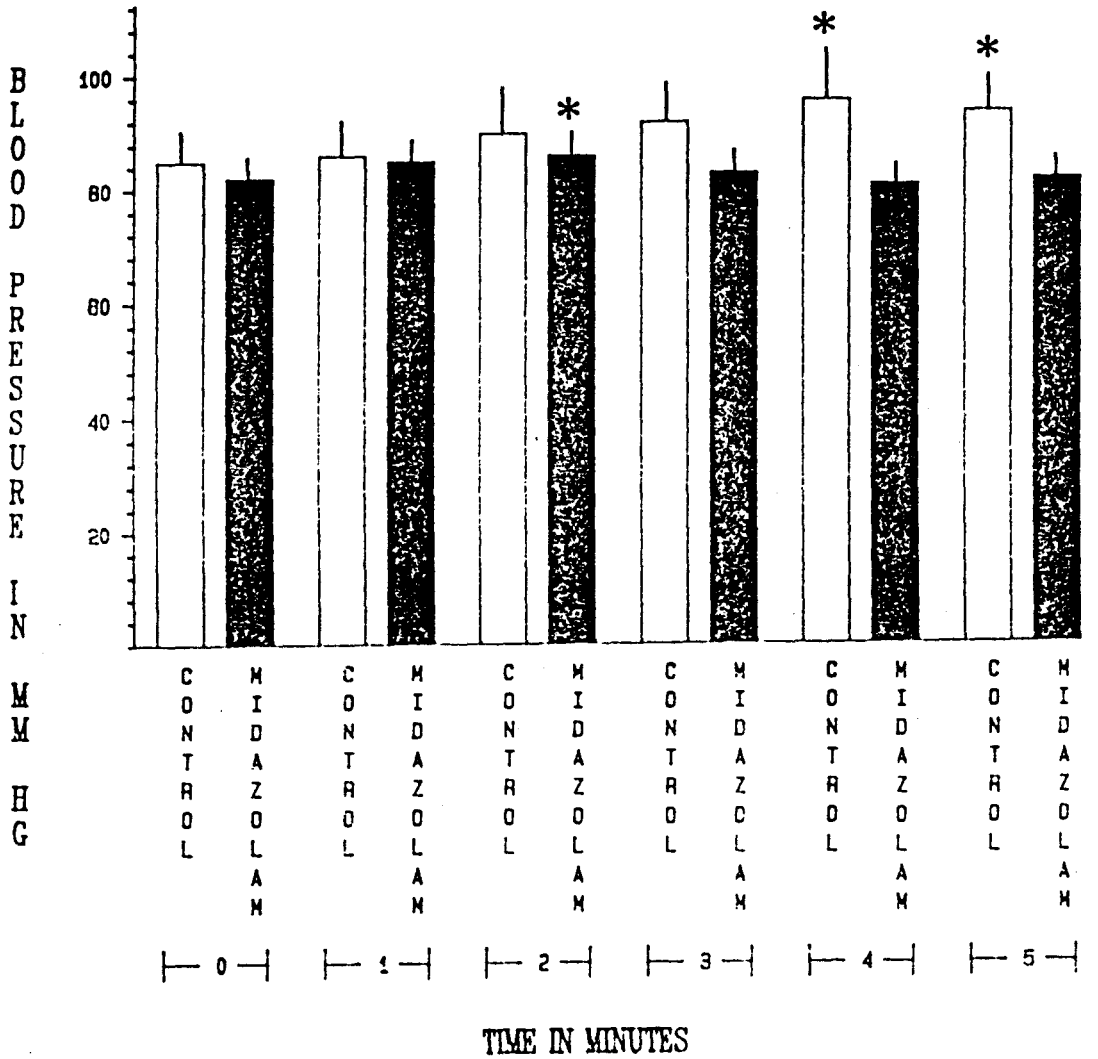
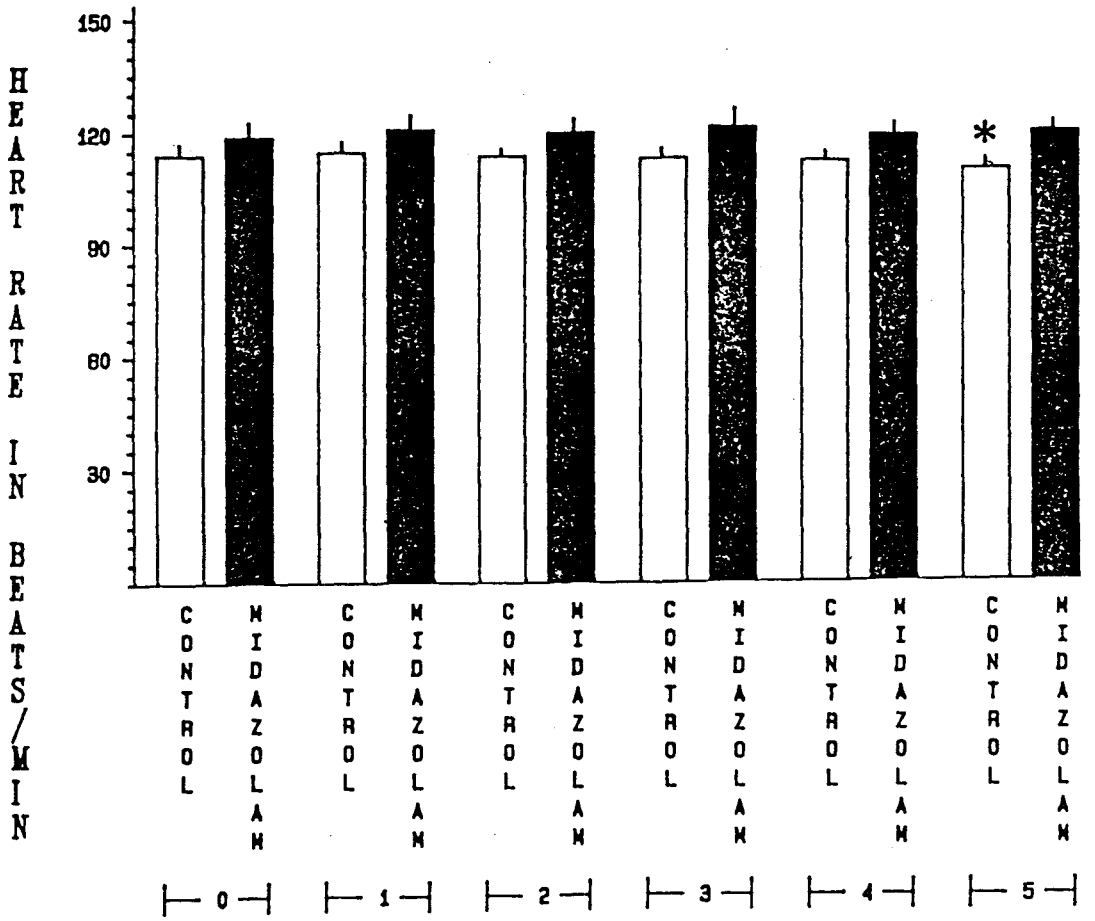

BLOOD PRESSURE IN MM HG
100
80
60
40
20
*
*
CONTROL
MIDAZOLAM
0
1
2
3
4
5
TIME IN MINUTES

Figure 13. The effect of midazolam on heart rate prior to placement of the retractor between the 5th and 6th ribs. Timing began immediately before skin incision and continued for five minutes. The asterisk indicates a significant difference ($p<.05$) between the indicated value and its corresponding preincision control value.


HEART RATE IN BEATS/MIN
150
120
90
60
30
*
CONTROL
MIDAZOLAM
0
1
2
3
4
5
TIME IN MINUTES

Midazolam-Treated Animals

The changes in heart rate and mean arterial blood pressure in the first 5 minutes following skin incision are shown in Figures 12 and 13. Blood pressure in the midazolam group showed a significant increase 2 minutes after skin incision and then slowly returned to the preincision control level. Heart rate, like blood pressure, was somewhat more stable in the midazolam group. In fact, the maximum deviation from from the preincision control level was 2 beats/minute, and at no time did heart rate change significantly from preincision control levels.

ANOVA

An analysis of variance was used to compare the control and midazolam groups with respect to blood pressure and heart rate. The results showed a significant time effect and a nonsignificant drug effect for both blood pressure and heart rate. Only blood pressure showed a significant drug*time interaction, and this reflects the increased blood pressure in the control group and decreased blood pressure in the midazolam group in the 5 minutes following skin incision. The significant time effect taken with the insignificant drug effect suggests that although there were changes in the control and midazolam groups in both heart rate and

blood pressure during the 5 minutes following skin incision, the groups did not differ significantly from each other with regard to these two hemodynamic variables.

CHAPTER IV

DISCUSSION

Highlights of the Results

Stress Responses in Anesthetized Control Animals

This study was undertaken to investigate changes in plasma catecholamines, cortisol and renin activity and several hemodynamic variables following either a hypotensive stress or surgical trauma. Further studies then examined whether three benzodiazepines could attenuate these stress responses.

Hemodynamic Variables

Hypotensive Stress. Sodium nitroprusside, ***Nipride***, was used to produce the hypotensive stress in Experiments I and II. The hypotensive effect of this drug is immediate and ends within minutes after termination of the infusion. Nitroprusside is rapidly metabolized to cyanide and subsequently converted to thiocyanate (Roche Laboratories, 1982).

Nitroprusside is a potent vasodilator that acts directly on the smooth muscle of both resistance and capacitance vessels (Nickerson and Ruedy, 1975). Nitroprusside does not appear to have any direct cardiac effects (Chiba et al., 1982). Most effects on measurements of cardiac performance appear to be secondary to the reduction in peripheral resistance and venous tone, which alter afterload and preload, respectively (Palmer and Lasseter, 1975). Also, nitroprusside has not been shown to have any effects on the autonomic or central nervous systems, and there is no evidence that its effects are mediated by these systems (Palmer and Lasseter, 1975).

Although blood pressure is decreased in both dogs and humans following nitroprusside administration, the overall pattern of cardiovascular responses is inconsistent. Adams (1974) and Rowe and Henderson (1975) reported that heart rate was significantly increased in anesthetized dogs, but decreases and only slight increases have also been reported (Chiba et al., 1982). Cardiac output was found to be increased (Rowe and Henderson, 1975) and unchanged (Adams, 1974). Stroke volume was shown to be decreased (Adams, 1974) or increased (Rowe and Henderson, 1975). Decreases in systemic vascular resistance index, wedge pressure and right atrial pressure have been reported (Adams, 1974; Rowe and

Henderson, 1975). In unanesthetized normotensive and hypertensive human patients, nitroprusside produced a marked lowering of arterial blood pressure, slight increase in heart rate, mild decrease in cardiac output, and moderate diminution in calculated total peripheral vascular resistance (Roche Laboratories, 1982).

In the present study, the hemodynamic profile of the dogs remained relatively stable during the nitroprusside infusion. Cardiac index and stroke work index, the only variables other than arterial blood pressure to change significantly during the infusion, were significantly decreased both during the infusion and on recovery. The decreases found in cardiac index and stroke work index are probably due to the statistically significant decreases in systemic vascular resistance index reflecting arteriolar vasodilation. Statistically nonsignificant decreases in heart rate, right atrial pressure and wedge pressure were also found.

The lack of many significant cardiovascular responses in the control animals probably was related not only to the effects of nitroprusside, but also to the enflurane-nitrous oxide-oxygen anesthesia used in this study. Enflurane often causes decreases in systemic vascular resistance index and cardiac output, but cardiac rhythm generally remains stable (Hall and Boulton, 1979). The addition of nitrous oxide to

enflurane anesthesia is reported not to increase the cardiovascular depressant effects of enflurane, but rather to reduce the magnitude of the decrease in heart rate and blood pressure (Smith et al., 1978).

Surgical Stress. The animals in Experiment III were stressed by skin incision and thoracotomy. Numerous studies have documented hemodynamics and catecholamine responses to both abdominal and thoracic surgery. Hoar et al. (1980b) and Kim et al. (1981) reported hemodynamic reponses to sternotomy in patients undergoing myocardial revascularization. Sternotomy and cardiopulmonary bypass cause the greatest overall sympathetic stimulation in surgical patients. Although anesthesia was maintained with halothane-nitrous oxide-oxygen by Hoar et al. and with enflurane-nitrous oxide-oxygen by Kim et al., both of these groups found increases in heart rate, mean arterial blood pressure, and systemic vascular resistance index following sternotomy.

In the present study, skin incision and rib spread caused statistically significant decreases in mean arterial blood pressure, heart rate, cardiac index and stroke work index were found. Systemic vascular resistance index increased, but this was not statistically significant. The results of this study and those of Hoar et al. (1980b) and Kim et al. (1981) demonstrate that hemodynamics and sympa-

thetic responses relate to the intesity of the stresses. While ribs were spread in this study, they were not broken, and thus would not have provided as strong a stress as the sternotomy in the Hoar et al. and Kim et al. studies. Also, Hoar et al. (1980b) administered morphine sulfate, scopolamine, thiopental and curare to their patients prior to the sternotomy; and Kim et al. (1981) administered morphine sulfate, scopolamine, thiopental, succinylcholine and pancuronium to their patients. However, the only drug other than the anesthetic mixture administered in the present study was thiopental on induction of anesthesia.

Catecholamines

The nitroprusside-induced hypotension resulted in statistically significant increases in plasma epinephrine and norepinephrine.

Steriods have been reported to inhibit the extraneuronal uptake of catecholamines in the rat heart (Iverson and Salt, 1970) and in smooth muscle (Nicol and Rae, 1972). An inhibition of the normally rapid inactivation of catecholamines by tissue uptake would be expected to produce higher concentrations of catecholamines in the vicinity of adrenergic receptors. This, in turn, could produce exaggerated and prolonged pharmacological responses.

Nicol and Rae (1972) reported 50% inhibition of catecholamine uptake 2 in peripheral arteries with plasma cortisol levels of 69.2 ng/ml; much less than required to inhibit uptake 2 in heart (Iverson and Salt, 1970). In the present experiments, cortisol levels in control animals increased during the stress. Peak levels, above 70 ng/ml, were seen 10 and 20 minutes following the surgical and hypotensive stresses, respectively. In these same animals, catecholamine levels rapidly increased, within two minutes, in response to stress and tended to remain elevated. Even though some degree of uptake 2 inhibition may have been present, the lack of an association between peak cortisol and catecholamine levels, 2 minutes vs 10 minutes, suggests that cortisol was not a major determinant of the early rise in catecholamine levels and pharmacological actions *in vivo* following hypotensive or surgical stresses.

The stress response to the thoracotomy procedure, while showing the same trend as the hypotensive stress, was considerably milder.

Based on the results of previous clinical studies, (Anton, 1964; Halter et al.,1977; Balasaraswathi et al., 1978 and 1980; Madsen et al., 1978; Tan et al., 1978; Hoar et al., 1980a and 1980b; Kim et al., 1981) it was assumed

that surgical stress such as thoracotomy used in this study would be a potent stimulant to plasma catecholamine levels. Although a large increase in both epinephrine and norepinephrine were seen following the thoracotomy procedure, these increases were not statistically significant.

Because most of the previous studies showing an increase in catecholamines in response to thoracotomy used patients with myocardial disorders, it might seem that there is a marked difference in the physiologcial response to surgical stress in patients with and without cardiovascular disorders. However, Barta et al. (1976) measured the release of dopamine-beta-hydroxylase, an enzyme involved in norepinephrine synthesis, into coronary blood after thoracotomy and during cardiopulmonary bypass in patients undergoing correction of a mitral valve defect. Their results suggested that while thoracotomy alone was not a stimulus that would lead to increased sympathetic activity in the heart, the cardiopulmonary bypass surgery led to considerable sympathetic activity in the heart. These results may however reflect a difference between cardiac and generalized sympathetic activation. In studies of patients with cardiovascular disorders, some researchers show increased sympathetic activity in response to certain surgical stresses while others do not. Consequently, the inability of the control cat-

echolamine data to reach statistical significance following rib spread was most likely due to the large amount of variability in the data, and reflects the variability of the plasma catecholamine response to the thoracotomy procedure. The possibility also exists that the sympathetic response of individuals to surgical stress does not conform to a parametric statistical analysis.

Cortisol

Cortisol was significantly elevated in response to both the hypotensive and surgical stresses. Although the maximum amount of elevation during the hypotensive stress (73%) was greater than that during the surgical stress (38%), elevations in cortisol generally were of similar magnitude during both stresses. Since the magnitude of the catecholamine and renin response to the hypotensive stress was considerably greater than that of the thoracotomy stress, it would seem that while catecholamines and renin activity are quite sensitive to alterations in blood pressure, cortisol secretion is elicited by a wider variety of stressful situations.

Plasma Renin Activity

The effect of surgical stress on plasma renin activity was markedly less than that of the hypotensive stress. Plasma renin activity, which increased 134% over the pre-stress control level during the hypotensive stress, showed nonsignificant increases following rib spread and only reached significance with a 49% increase 20 minutes after removal of the retractor. Unlike the catecholamine data, the small rise in plasma renin activity following thoracotomy cannot be attributed to excessivie variability in these data; however, the moderate increase in plasma renin activity may be related to the smaller degree of elevated catecholamines, since sympathetic nerve activity is one stimulus to renin release.

A second reason that plasma renin activity was not as elevated following the surgical stress is that during the thoracotomy procedure, blood pressure, which was lowered 30% during the hypotensive stress, only decreased a maximum of 16% following surgical trauma; consequently, afferent renal artery blood pressure, an important factor in renin release, probably was not sufficiently lowered to initiate a large release of renin.

Finally, alterations in fluid balance, another important factor in renin release, were probably not a factor in

either stress-producing procedure, since fluid balance was maintained by careful monitoring of fluid intake and output.

Stress Reduction by Benzodiazepines

Hemodynamic Variables

There were no outstanding differences between the benzodiazepine and control groups or between any of the three benzodiazepine groups during the hypotensive stress. In fact, the only noteworthy hemodynamic change was a statistically significant decrease in heart rate and right atrial pressure seen following midazolam pretreatment; however, the magnitude of the decrease in both of these variables was not great. During the thoracotomy stress, the hemodynamic profile of animals pretreated with midazolam approximated that of control animals. The results suggest that with respect to hemodynamic variables, control animals and benzodiazepine-treated animals respond similarly to hypotensive and surgical stresses.

Catecholamines, Cortisol and Renin Activity

The results indicate that diazepam, midazolam and lorazepam are all capable of blunting the elevated plasma epinephrine, norepinephrine and cortisol levels seen in response to a hypotensive stress. Although plasma catecholamines and cortisol levels appeared to be decreased to a greater extent following diazepam pretreatment, the degree of suppression of midazolam and lorazepam was only slightly less than that of diazepam. On the other hand, none of the benzodiazepines were effective in suppressing stress-induced elevations in plasma renin activity. Although there are definite pharmacodynamic and chemical differences between diazepam, lorazepam and midazolam, the results of this study do not recommend one over the other for attenuation of stress responses of short duration.

Time Course of Midazolam's Actions during Hypotensive Stress

Because the ability of diazepam and lorazepam to suppress the hypotension-induced stress responses was similar to that of midazolam, the new water-soluble benzodiazepine, midazolam was chosen for further investigation. When midazolam was administered 1-1/2 hours prior to the hypotensive

stress, it was able to blunt both the norepinephrine and epinephrine stimulatory response to a hypotensive stress. However, cortisol levels remained elevated throughout the hypotensive and recovery periods.

In comparing these results with those from the experiment in which midazolam was given 4 minutes prior to the stress, it appears that midazolam effected a stronger suppression when given just before the stress. When midazolam was given 4 minutes prestress, the largest increase in epinephrine was statistically insignificant (40%) and norepinephrine decreased 14%. On the other hand, when midazolam was given 1-1/2 hours prestress, a 98% rise in epinephrine, which was statistically significant, was seen during the recovery period, and norepinephrine showed a nonsignificant 46% increase during the hypotensive period. Also, plasma cortisol levels, which were attenuated during the first 10 minutes of the hypotensive period when midazolam was given just prior to the stress, were not attenuated when midazolam was given 1-1/2 hours prestress. The entire time course of midazolam's actions on plasma catecholamines and cortisol were not determined in this study. The results suggest that midazolam is effective in suppressing the stress-induced surges in catecholamines for a longer duration of time than it is effective in suppressing stress-induced elevations in cortisol.

Effects of Midazolam on Surgical Stress Responses

In the thoracotomy experiment, as in the hypotensive experiment, midazolam was effective in reducing the stress-induced elevations in cortisol levels, but did not affect plasma renin activity. The catecholamine data in control animals in the thoracotomy experiment were not as markedly elevated as they were in the control animals in the hypotensive experiment. Also, there was a large amount of variability in the catecholamine data from control dogs that was not seen in midazolam-treated dogs. Thus, it is possible that midazolam was effective in counteracting surgery-induced elevations in catecholamines in those instances where the subject was predisposed to stress-induced elevations of catecholamines.

Summary

One might speculate on the potential clinical benefits of benzodiazepines on stress-induced elevations in plasma catecholamines, cortisol and renin activity in patients. The data from this animal study indicate that when a situation, such as drug-induced hypotension or surgical trauma,

is expected to result in levated plasma catecholamines, cortisol and renin activity then benzodiazepines could be of value in obtunding the catecholamine and cortisol, but not renin activity, stress response. Moreover, when an elevation in catecholamines cannot be definitely predicted, in a situation analogous to the thoracotomy procedure, benzodiazepines might also be effective in inhibiting potential rises in plasma catecholamines. It is anticipated that controlled clinical studies will establish the similarities of patient responses to benzodiazepines with the reponses obtained in this study.

Anesthetic Actions of Benzodiazepines

Since all of the benzodiazepines used in this study are capable of inducing anesthesia, it might seem reasonable to assume that the stress-inducing actions of benzodiazepines merely reflected an increased depth of anesthesia. In other words, the attenuation in the adrenergic and cortisol response to surgical or hypotensive stress by benzodiazepine premedication may have resulted from a generalized depression caused by the addition of a second anesthetic agent (benzodiazepines) to the ongoing enflurane-nitrous oxide-oxygen anesthesia.

However, results from a pilot study done in this laboratory demonstrated that using nitrous oxide to increase the depth of anesthesia to the level to which the benzodiazepines might have increased it did not blunt the hypotension-induced rise in epinephrine and norepineprhine. This study used the Melvin et al. (1980) criteria to approximate the increased depth of anesthesia that the dose of midazolam used in our study (0.2 mg/kg) would yield. The Melvin group found that midazolam reduced the halothane MAC in a dose-dependent manner, and extrapolating from their results to the dose of midazolam used in our study, midazolam would appear to decrease the volume percent of enflurane to achieve

enflurane MAC by 10%. In the pilot study, the anesthetic delivered was increased from the 1.3 MAC used in the present study to 1.5 MAC (a 15.4% increase). This was a slightly greater increase than that predicted by the results of the Melvin et al. study. The amount of nitrous oxide that would cause this multiple of MAC was calculated to be 40% in 60% oxygen.

One subject in the pilot study showed a 102% increase in norepinephrine and another subject showed a 105% increase in norepinephrine. Thus, even though the benzodiazepines may have slightly increased the depth of anesthesia, the response of plasma catecholamines to stress appears to be active at the level of anesthesia used in the pilot study. Consequently, the attenuation of stress-induced elevations in plasma epinephrine, norepinephrine, and cortisol found in benzodiazepine-treated animals in the present study does not appear to be due to a generalized anesthesia-induced depression.

In addition, the intravenous administration of 0.2 mg/kg midazolam to awake dogs in the 1-1/2 hour pretreatment study caused only minimal observable changes in the dog's behavior. The dogs remained awake and responsive, although they appeared less anxious. In a purely subjective sense the dogs could have been characterized as sedated.

Pharmacological Basis of the Results

Overview of Benzodiazepine Receptors

Peripheral versus Central Site of Action

The benzodiazepines could have attenuated the stress responses in the study by acting at a site either within or outside of the central nervous system. Benzodiazepines have been reported to bind specifically to both central and peripheral sites (Braestrup & Squires, 1977; Mohler and Okada, 1977; Davies and Huston, 1981). However, benzodiazepine binding to kidney, liver and lung tissue is pharmacologically distinct from binding to brain tissue, with the peripheral binding sites showing significantly lower affinities for benzodiazepines than central sites (Braestrup and Squires, 1977). Furthermore, the displacement of ^{3}H-diazepam from central binding sites by clinically active benzodiazepines correlated well with an *in vitro* anti-anxiety test and with recommended clinical doses (Braestrup et al., 1977). No such correlation, however, was found for peripheral sites (Braestrup and Squires, 1977). The results of these pharmacological studies thus suggest that periph-

eral binding sites do not mediate the clinical effects of benzodiazepines.

Electrophysiological studies also do not support a peripheral site of action for the benzodiazepines. Diazepam could only inhibit nictitating membrane contraction or pupil dilation following preganglionic cervical nerve stimulation at doses well above therapeutic concentrations (Chai and Wang, 1966; Sigg et al., 1971). Furthermore, no dose of diazepam affected postganglionic stimulation in the Chai and Wang study. However, diazepam was effecive in inhibiting centrally evoked sympathetic responses in both of these studies. Consequently, results from both pharmacological and electrophysiological studies would suggest that the benzodiazepines used in this study most likely would have acted at sites within rather than outside of the central nervous system to attenuate the stress-induced stimulatory responses seen in this study.

Benzodiazepine Receptors in the Central Nervous System

Benzodiazepine receptors are well distributed throughout the central nervous system. Studies of both human and rat brains have indicated that the frontal cortex, occipital

cortex, limbic forebrain and hippocampus contain the highest density of benzodiazepine binding sites; hypothalamus, cerebellum and corpus striatum show a moderately high density of binding sites; and the pons and medulla contain the lowest density of binding sites (Braestrup and Squires, 1977; Braestrup et al., 1977). Because benzodiazepine binding to these central sites is saturable and specific, occurs with high affinity and has a good correlation between the *in vivo* potency and binding strength, the central benzodiazepine binding sites are considered to be pharmacologically relevant receptors (Braestrup and Squires, 1978; Speth et al., 1980).

Although the early studies of Squires and Braestrup (1977) indicated that only one class of benzodiazepine receptors exists in the central nervous system, more recent studies have suggested that there are actually two classes of benzodiazepine receptors, which can be differentiated by the binding of a new class of agents, the triazolopyridazines (Squires et al., 1979; Lippa et al., 1979) In fact, Klepner et al. (1979) have suggested that both benzodiazepines and triazolopyridazines bind to Type I benzodiazepine receptors and mediate anxyolytic actions. In contrast Type II benzodiazepine receptors appear only to bind to benzodiazepines with high affinity, are considered to be coupled

to GABA (gamma-aminobutyric acid) receptors and mediate nonanxyolytic pharmacological effects.

Thus, after considering the current state of knowledge of benzodiazepines, it can be assumed that the benzodiazepines in the present study attenuated stress responses by acting at central Type II benzodiazepines receptors.

Benzodiazepine-GABA-Chloride Ionophore Receptor Complex

A large body of evidence suggests that many of the pharmacological effects of benzodiazepines can be attributed to their actions on GABA systems in the brain. In 1975 Haefely et al. proposed that GABA might be involved in the central actions of benzodiazepines since benzodiazepines' anticonvulsant, muscle relaxant, ataxic and sedative effects, but not their anxyolytic effects, resemble accepted or presumed functions of GABA. Haefely et al. also showed that benzodiazepines could counteract convulsions associated with a partial inhibition of brain GABAergic transmission, enhance GABA-mediated presynaptic inhibition in the spinal cord and cuneate nucleus, and facilitate GABAergic postsynaptic inhibition in the substantia nigra.

Costa et al. and Fuxe et al. also suggested in 1975 that GABA was involved in the action of benzodiazepines. Costa et al. showed that benzodiazepines could reduce the increase in cerebellar cyclic GMP content generated by a decrease in GABAergic transmission, and Fuxe et al. showed that although benzodiazepines could reduce central dopamine and norepinephrine turnover, these effects could be explained by an increase in GABA-receptor activity.

Further evidence for a benzodiazepine-GABA interaction comes from Enna (1979) who found a very high correlation between the regional distribution of ^{3}H-benzodiazepine binding sites and ^{3}H-GABA binding sites. Also, Tallman et al. (1978) have shown that while GABA and the GABA agonist muscimol could increase the affinity of benzodiazepines for their binding sites without changing the number of binding sites, this effect could be blocked by bicuculline, a competitive GABA antagonist. Thus, at the present time it is generally agreed that GABA is intimately involved in many actions of the benzodiazepines.

In addition to the postulated benzodiazepine-GABA interaction, benzodiazepines appear to interact with a chloride ionophore. Martin and Candy (1980) have shown that increasing chloride ion concentrations facilitate benzodiazepine binding. It has also been shown that GABA-medi-

ated depolarization is due to increased chloride conductance (Nishi et al., 1974). Thus, a close linkage between the benzodiazepine receptor, GABA receptor and chloride ionophore has been proposed by Costa and Guidotti (1979), Gallager et al., (1980), Martin and Candy (1980) and Paul et al. (1981).

According to this supramolecular theory of benzodiazepine action, GABA occupation of the postsynaptic receptor opens the chloride ionophore allowing chloride ions to pass through the membrane according to the concentration gradient. A chloride ion flux into a cell results in hyperpolarization of the cell membrane, a reduction in the response of the cell to incoming excitatory stimuli and postsynaptic inhibition. On the other hand, a chloride flux from a nerve cell terminal results in depolarization of the nerve terminal, a reduction in the amount of neurotransmitter released from the cell and presynaptic inhibition. Benzodiazepines are thought to act by enhancing this GABA-induced increase in chloride conductance.

In summary, benzodiazepine receptors appear to be part of a supramolecular complex consisting of a benzodiazepine receptor, GABA receptor and chloride ionophore. Benzodiazepines appear to mediate their effects by enhancing the GABAergic increase in chloride conductance. Although the

present study was not designed to further investigate the supramolecular complex as the mechanism of benzodiazepine action, this theory does allow for a better understanding of some of the effects of benzodiazepines seen in this study.

Central Benzodiazepine Receptors and Hypotensive Stress

Central Benzodiazepine Actions and Catecholamines

Plasma epinephrine and norepinephrine levels were significantly elevated following the hypotensive stress, and each of the benzodiazepines used in this study effectively attenuated the response of the catecholamines to this stress. Assuming that the benzodiazepines do indeed act at central rather than peripheral sites, then the effects of benzodiazepines on plasma catecholamine levels would be reflective of a primary action within the central nervous system. The benzodiazepines do not appear to have acted at the level of the medulla, because these drugs have been shown to be either ineffective inhibitors of pressor or depressor responses obtained by stimulation of the medulla (Antonaccio and Halley, 1975) or only slightly effective

inhibitors of these responses (Chai and Wang, 1966). Although this is not surprising considering that the medulla is only sparsely populated with benzodiazepine receptors (Braestrup et al., 1977; Braestrup and squires, 1977; Mohler and Okada, 1977), this is an instance of dissociation between GABA and benzodiazepine actions.

More likely sites of benzodiazepine actions would include the hypothalamus and other supramedullary structures involved in sympathetically mediated functions. An early study by Chai and Wang (1966) showed that the hypothalamus was considerably more susceptible than the medulla or peripheral sites to diazepam's centrally mediated depressant effects. Sigg et al. (1971) subsequently showed that diazepam could inhibit a hypothalamically evoked vasopressor response. Further evidence supporting a hypothalamic site of the sympathetic actions of benzodiazepines comes from binding studies. Several investigators have reported a high concentration of binding sites in the hypothalamus (Braestrup et al., 1977; Braestrup and Squires, 1977; Mohler and Okada, 1977). Furthermore, GABA has been shown to be involved in sympathetically mediated cardiovascular responses and a forebrain site of action has been implicated by DiMicco et al. (1977). In fact, Anotnaccio and Halley (1975) have shown that benzodiazepines can mediate autonomic

activity at such caudal areas as the rostral periaqueductal grey and dorsal tegmentum. Antonaccio (1979) later suggested that these more caudal areas may help form tracts that synapse with bulbospinal tracts and then connect with preganglionic neurons to mediate sympathetic activity. Consquently, benzodiazepines do not appear to act exclusively at the hypothalamus, but may act throughout well-defined supramedullary centers.

The means by which benzodiazepines regulate peripheral sympathetic activity can be considered by examining the effects of benzodiazepines on central catecholamines. Corrodi et al. (1971) have suggested that the inhibitory effect of benzodiazepines on stress-induced increases in norepinephrine turnover may be due to a reduction in nervous impulse flow, and their suggestion is supported by studies by Taylor and Laverty (1973) and Lidbrink and Farnebo (1973). Taylor and Laverty showed that benzodiazepines had no significant effect on endogenous catecholamine levels throughout the rat brain, on the uptake of catecholamines into catecholaminergic neurons or on catecholamine metabolism. In the Lidbrink and Farnebo study, the authors used cerebral cortex slices from rat brains to confirm that benzodiazepines are ineffective inhibitors of norepinephrine uptake and to further show that benzodiazepines do not

affect catecholamine release. Thus, the effects of benzodiazepines on the stress-induced increases in plasma norepinephrine and epinephrine seen in the present study most probably were due to a decrease in neuronal activity and involved supramedullary sites of the central nervous system.

On the other hand, because of the wide distribution of both GABA and benzodiazepine receptors within the central nervous system, and because other central nervous system areas such as the cortex and limbic system may affect sympathetically mediated functions (Antonaccio, 1977), benzodiazepines may act at areas other than the hypothalamus. Thus, benzodiazepines appear to attenuate cardiovascular responses at a site caudal to the medulla, possibly involving the hypothalamus, and the sympathetic effects of benzodiazepines may be related to GABAergic actions.

Benzodiazepines and Stress-Induced Elevations in Cortisol

Plasma cortisol levels were significantly elevated in response to both the hypotensive and surgical stresses, and this response was attenuated by the benzodiazepines. Although the specific means by which the benzodiazepines produced this effect cannot be determined from this study,

possible explanations can be entertained by considering reported effects of benzodiazepines and GABA on the cortisol secretory process.

Plasma cortisol levels are determined by a complex interplay between the hypothalamus, anterior pituitary and adrenal cortex (Guyton, 1976). Cortisol is secreted from the adrenal cortex, and the rate of secretion is controlled almost entirely by adrenocorticotropic hormone (ACTH). ACTH secretion is in turn thought to be primarily controlled by a hypothalamic releasing factor, corticotropin releasing factor (CRF). In addition, there is also a direct inhibitory feedback of cortisol on the hypothalamus and anterior pituitary. Thus, continuing with the previously discussed assumption that benzodiazepines have a central rather than peripheral site of action, the attenuation of the plasma cortisol stress response would most likely have occurred at the level of either the hypothalamus or anterior pituitary.

Because the effects of benzodiazepines on CRF and ACTH secretion are not well defined, it might be useful to examine the role of GABA, the neurotransmitter thought to mediate the actions of the benzodiazepines, in the hypothalamus and anterior pituitary. Makara and Stark (1974) showed that while GABA infused into the third ventricle of rats could block a surgery-induced increase in plasma corticosterone

levels, picrotoxin, a GABA antagonist, increased plasma corticosterone levels. Futhermore, this effect held even when the hypothalamus was deafferentated, leading these authors to suggest that GABA of hypothalamic origin might be involved in regulating ACTH secretion. Also, GABA has been located in both the anterior pituitary and the hypothalamus (Racagni et al., 1981). Moreover, Tappaz et al. (1981) have presented evidence that a hypothalamic GABAergic pathway may run from the arcuate nucleus to the median eminence and they suggest that GABA may act directly on the anterior pituitary. Indications that GABA may act directly on the anterior pituitary come from the identification of GABA binding sites in the anterior pituitary of both rat and human brains (Grandison, 1981) and from studies that have shown that GABA can act on the anterior pituitary to prevent the release of prolactin, another anterior pituitary hormone (Cocchi et al., 1981; Grandison, 1981; McCann et al., 1981). Furthermore, benzodiazepines have been shown to act at the level of the anterior pituitary to potentiate the GABA-induced decrease in prolactin (Grandison, 1981).

In summary, it is most likely that the benzodiazepines decreased the cortisol stress response in this study by acting at the level of the hypothalamus and pituitary. Although there is evidence suggesting that both GABA and

benzodiazepines can act directly on the anterior pituitary, benzodiazepine receptors have not yet been located in conjunction with the proposed anterior pituitary GABA receptors. Furthermore, although evidence showing that GABA, and even benzodiazepines, can act directly on the anterior pituitary, benzodiazepine receptors have not yet been located in conjunction with the proposed anterior pituitary GABA receptors. Also, evidence showing that GABA, and even benzodiazepines, can act at the anterior pituitary to prevent prolactin release does not prove that GABA and benzodiazepines act at the anterior pituitary to inhibit ACTH release. Alternatively, the benzodiazepines and GABA could act at the level of the hypothalamus and inhibit CRF or some similar inhibitory substance. Consequently, although it seems likely that the benzodiazepines in the present study acted along the hypothalamic-pituitary axis, the exact site of action cannot be determined.

Benzodiazepines and Elevations in Plasma Renin Activity

Plasma renin activity was significantly elevated in response to the hypotensive stress. However, contrary to the attenuation of stress-induced elevations in plasma cate-

cholamines and cortisol levels by benzodiazepines, these drugs were unable to affect the stress-induced rise in plasma renin activity. Renin is released from the juxtaglomerular apparatus of the kidney in response to increased sympathetic nerve activity, reduced glomerular filtration rate or vasoconstriction of the renal arteries (Sweet, 1977). Considering that these stimuli are reflective of peripheral events and benzodiazepines act primarily at central sites, it is not surprising that benzodiazepine pretreatment did not attenuate the stress-induced elevation in plasma renin activity. Also, although benzodiazepine binding sites have been found in the kidney, these binding sites are pharmacologically distinct from the central binding sites (Braestrup and Squires, 1977). Thus, it can be assumed that the inability of benzodiazepines to block the rise in plasma renin activity seen in response to a hypotensive stress is due to the fact that the benzodiazepines act centrally rather than peripherally.

Summary

The ability of benzodiazepines to attenuate stress-induced elevations in plasma epinephrine, norepinephrine and

cortisol, but not renin activity, is of pharmacological as well as clinical interest. Although the present study was not designed to investigate the mechanism and site of the stress-reducing effects of the benzodiazepines, current literature suggests that the benzodiazepines act at central Type II benzodiazepine receptors, often in conjunction with GABA. The inhibitory effects of the benzodiazepines on stress-induced elevations of catecholamines and cortisol appear to have been mediated at a supramedullary site, possibly at the level of the hypothalamus or pituitary. On the other hand, the benzodiazepines were ineffective in attenuating stress-induced elevations in plasma renin activity, and this is considered to be related to the central rather than peripheral site of benzodiazepine actions. Further studies, of course, are needed to correlate the clinical effects of benzodiazepines with their concomitant pharmacological actions.

Significance of this Research to Health Care

A variety of stressful situations can lead to elevated plasma catecholamines, cortisol and renin activity, which may at times be undesirable. For example, in cardiac patients with ischemic heart disease the physician may wish to avoid some of the potential effects of high plasma catecholamine and renin activity levels -- increased afterload and ventricular wall tension -- which can lead to increased oxygen demand. Moreover, it may be beneficial to avoid elevated plasma cortisol levels in some patients with hyperadrenocortical function or with labile diabetes.

In this study, three benzodiazepines attenuated stress-induced elevations in plasma epinephrine, norepinephrine, and cortisol levels in anesthetized dogs. Further studies are needed to determine whether similiar actions occur in human patients.

BIBLIOGRAPHY

Adams, A.P., Clarke, T.N.S., Edmonds-Seal, J., Foex, P., Prys-Roberts, C., And Roberts, J.G. The Effects of Sodium Nitroprusside on Myocardial Contractility and Haemodynamics. *Br. J. Anaesth.*, *46*: 807-817, 1974.

Abukhres, M.M., Ertel, R. J., Dixit, B. N. and Vollmer, R. R. Role of the Renin-Angiotensin System in the Blood Pressure Rebound to Sodium Nitroprusside in the Conscious Rat. *Eur. J. Pharmacol.*, *58*: 247-254, 1979.

Allonen, H., Ziegler, G., and Klotz, U. Midazolam Kinetics. *Clin. Pharmacol. Ther.*, *30*: 653-661, 1982.

Alps, B. J., Harry, T. V. and Southgate, P. J. The Pharmacology of Lorazepam, a Broad-Spectrum Tranquillizer. *Curr. Med. Res. Opin.*, *1*: 239-261, 1973.

Ameer, B. and Greenblatt, D. J. Lorazepam: A Review of Its Clinical Pharmacological Properties and Therapeutic Uses. *Drugs*, *21*: 161-200, 1981.

Anton, A. H., Gravenstein, J. S. and Wheat, M. W. Extracorporeal Circulation and Endogenous Epinephrine and Norepinephrine in Plasma, Atrium and Urine in Man. *Anesthesiology*, *25*: 262-269, 1964.

Antonaccio, M. J. Pharmacology of Central Mechanisms Governing the Circulation. In M. J. Antonaccio (Ed.), *Cardiovascular Pharmacology*. New York: Raven Press, 1977.

Antonaccio, M. J. Cardiovascular Pharmacology of Anxiolytics. In S. Fielding and H. Lal (Eds.), *Anxiolytics*. Mount Kisco, New York: Futura Publishing Company, 1979.

Antonaccio, M. J. and Halley, J. Inhibition of Centrally-Evoked Pressor Responses by Diazepam: Evidence for an Exclusive Supramedullary Action. *Neuropharmacology* *14*: 649-657, 1975.

Arkin, H. and Colton, R. R. *Statistical Methods*. New York: Barnes and Noble Books, 1970.

Bailey, D. R., Miller, E. D., Kaplan, J. A. and Rogers, P.W. The Renin-Angiotensin-Aldosterone System During Cardiac Surgery with Morphine-Nitrous Oxide Anesthesia. *Anesthesiology, 42*: 538-544, 1975.

Balasaraswathi, K., Glisson, S. N., El-Etr, A. and Azad, C. Effect of Priming Volume on Serum Catecholamines During Cardiopulmonary Bypass. *Can. Anaesth. Soc. J., 27*: 135-139, 1980.

Balasaraswathi, K., Glisson, S. N., El-Etr, A. and Mummaneni, N. Hemodynamic and Catecholamine Response to Isoflurane Anaesthesia in Patients Undergoing Coronary Artery Surgery. *Can. Anaesth. Soc. J., 29*: 533-538, 1982.

Balasaraswathi, K., Glisson, S. N., El-Etr, A. A. and Pifarre, R. Serum Epeinephrine and Norepinephrine During Valve Replacement and Aorta-Coronary Bypass. *Can. Anaesth. Soc. J., 25*: 198-203, 1978.

Barman, S. M. and Gebber, G. L. Pictrotoxin- and Bicuculline-Sensitive Inhibition of Cardiac Vagal Reflexes. *J. Pharmacol. Exp. Ther., 209*: 67-71, 1979.

Barta, E., Kvetnansky, R., Kuzela, L., Babusikova, F. and Mikulaj, L. Coronary A-V Difference of Plasma Dopamine-Beta-Hydroxylase Activity During Stress Situation of Cardiopulmonary Bypass in Patients. In E. Usdin, R. Kvetnansky and I. J. Kopin (Eds.), *Catecholamines and Stress*. Oxford: Pergamon Press, 1976.

Berne, R.M. and Levy, M.N. *Cardiovascular Physiology*. St. Louis: The C.V. Mosby Company, 1977.

Braestrup, C., Albrechtsen, R., and Squires, R. F. High Densities of Benzodiazepine Receptors in Human Cortical Areas. *Nature, 269*: 702-704, 1977.

Braestrup, C. and Squires, R. F. Specific Benzodiazepine Receptors in Rat Brain Characterized by High-Affinity ^{3}H-Diazepam Binding. *Proc Natl. Acad. Sci. USA, 74*: 3805-3809, 1977.

Braestrup, C. and Squires, R. F. Brain Specific Benzodiazepine Receptors. *Br. J. Psychiatry, 133*: 249-260, 1978.

Bromage, P. R., Shibata, H. R. and Willoughby, H. W. Influence of Prolonged Epidural Blockade on Blood Sugar and Cortisol Responses to Operations upon the Upper Part of the Abdomen and the Thorax. *Surg. Gynecol. Obstet., 132*: 1051-1056, 1971.

Buhler, F. R. Antihypertensive Beta-Blockade and the Renin-Angiotensin System. *Cardiology, 66* (Suppl. 1): 12-27, 1980.

Butler, M. J., Britton, B. J., Wood, W. G., Mainwaring-Burton, R. and Irving, M. H. Plasma Catecholamine Concentrations During Operation. *Br. J. Surg., 64*: 786-790, 1977.

Cairns, J. A. Hemodynamic Monitoring in Acute Myocardial Infarction. *Can. Med. Assoc. J., 121*: 905-910, 1979.

Chai, C. Y. and Wang, S. C. Cardiovascular Actions of Diazepam in the Cat. *J. Pharmacol. Exp. Ther., 154*: 271-280, 1966.

Chan, C.-C. and Kalsner, S. Termination of Reponses to Sympathetic Nerve Stimulation and to Noradrenaline in a Perfused Arterial Preparation: The Role of Neuronal and Extraneuronal Uptake. *J. Pharmacol. Exp. Ther., 222*: 731-740, 1982.

Chen, R. Y. Z., Fan, F.-C., Schuessler, G. B. and Chien, S. Baroflex Control of Heart Rate in Humans During Nitroprusside-Induced Hypotension. *Am. J. Physiol., 243* (Regulatory Integrative Comp. Physiol. 12): R18-R24, 1982.

Chiba, S., Kozu, T.M., and Watanabe, H. Analysis of Cardiac Actions on Nitroprusside in Intact Dogs and in Isolated Atria. *Jpn. Heart J., 23*: 613-621, 1982.

Cocchi, D., Casanueva, F., Locatelli, V. Apud, J., Martinez-Campos, A., Civati, C., Racagni, G. and Muller, E. E. Gabaergic Mechanisms in the Control of PRL and GH Release. In E. Costa, G. Di Chiara and G. L. Gessa (Eds.), *GABA and Benzodiazepine Receptors*. New York: Raven Press, 1981.

Cohen, P.J. Signs and Stages of Anesthesia. In L.S. Goodman and A. Gilman (Eds.), *The Pharmacological Basis of Therapeutics*. New York: Macmillan Publishing Co., Inc., 1975.

Conner, J. T., Katz, R. L., Bellville, J. W., Graham, C., Pagano, R. and Dorey, F. Diazepam and Lorazepam for Intravenous Surgical Premedication. *J. Clin. Pharmacol.*, *18*: 285-292, 1978.

Corrodi, H., Fuxe, K., Lidbrink, P. and Olson, L. Minor Tranquillizers, Stress and Central Catecholamine Neurons. *Brain Res.*, *29*: 1-16, 1971.

Cosgrove, D. O. and Jenkins, J. S. The Effects of Epidural Anaesthesia on the Pituitary-Adrenal Response to Surgery. *Clin. Sci. Mol. Med.*, *46*: 403-407, 1974.

Costa, E. and Guidotti. A. Recent Studies on the Mechanism Whereby Benzodiazepines Facilitate GABA-ergic Transmission. In P. Mandel and F. V. DeFeudis (Eds.), *GABA -- Biochemistry and CNS Functions*. New York: Plenum Press, 1979.

Costa E., Guidotti, A. and Mao, C. C. Evidence for Involvement of GABA in the Action of Benzodiazepines: Studies on Rat Cerebellum. In E. Costa and P. Greengard (Eds.), *Mechanism of Action of Benzodiazepines*. New York: Raven Press, 1975.

Cote, P., Gueret, P. and Bourassa, M. G. Systemic and Coronary Hemodynamic Effects of Diazepam in Patients with Normal and Diseased Coronary Arteries. *Circulation*, *50*: 1210-1216, 1974.

Cottrell, J. E., Illner, P., Kittay, M. J., Steele, J. M., Lowenstein, J. and Turndorf, H. Rebound Hypertension After Sodium Nitroprusside-Induced Hypotension. *Clin. Pharmacol. Ther.*, *27*: 32-36, 1980.

Daniell, H. B. Cardiovascular Effects of Diazepam and Chlordiazepoxide. *Eur. J. Pharmacol.*, *32*: 58-65, 1975.

Davies, L. P. and Huston, V. Peripheral Benzodiazepine Binding Sites in Heart and their Interaction with Dipyridamole. *Eur. J. Pharmacol.*, *33*: 209-211, 1981.

Delaney, T. J. and Miller, E. D. Rebound Hypertension After Sodium Nitroprusside Prevented by Saralasin in Rats. *Anesthesiology, 52*: 154-156, 1980.

DiMicco, J. A., Gale, K., Hamilton, B. and Gillis, R. A. GABA Receptor Control of Parasympathetic Outflow to Heart: Characterization and Brainstem localization. *Science, 204*: 1106-1109, 1979.

DiMicco, J. A., Hamilton, B. L. and Gillis, R. A. Central Nervous System Sites Involved in the Cardiovascular Effects of Picrotoxin. *J. Pharmacol. Exp. Ther., 203*: 64-71, 1977.

Dundee, J. W. New I.V. Anaesthetics. *Br. J. Anaesth., 51*: 641-648, 1979.

Dundee, J. W., McGowan, W. A., Lilburn, J. K., McKay, A. C. and Hegarty J. E. Comparison of the Actions of Diazepam and Lorazepam. *Br. J. Anaesth., 51*: 439-446, 1979.

Dundee, J. W., Samuel, I. O., Toner, W. and Howard, P. J. Midazolam: A Water-Soluble Benzodiazepine. *Anaesthesia, 35*: 454-458, 1980.

Elliott, H. W. Metabolism of Lorazepam. *Br. J. Anaesth., 48*: 1017-1023, 1976.

Engquist, A. Brandt, M. R., Fernandes, A. and Kehlet, H. The Blocking Effect of Epidural Analgesia on the Adrenocortical and Hyperglycemic Responses to Surgery. *Acta Anaesth. Scand., 21*: 330-335, 1977.

Enna, S. J. Regional Variation and Characteristics of GABA-Receptors in the Mammalian CNS. In P. Mandel and F. V. DeFeudis (Eds.), *GABA-Biochemistry and CNS Functions*. New York: Plenum Press, 1979.

Estafanous, F. G., Tarazi, R. C., Viljoen, J. F. and El Tawil, M. Y. Systemic Hypertension following Myocardial Revascularization. *Am. Heart J., 85*: 732-738, 1973.

Favre, L., Vallotton, M. B. and Muller, A. F. Relationship between Plasma Concentrations of Angiotensin I, Angiotensin II and Plasma Renin Activity During Cardiopulmonary Bypass in Man. *Eur. J. Clin. Invest., 4*: 135-140, 1974.

Forster, A. Utilisation of Midazolam as an Induction Agent in Anaesthesia. *Arzneimittelforsch., 31 (12a)*: 2243, 1981.

Forster,A., Gardaz, J. P., Suter, P. M. and Gemperle, M. I.V. Midazolam as an Induction Agent for Anaesthesia: A Study in Volunteers. *Br. J. Anaesth., 52*: 907-911, 1980.

Fragen, R. J., Gahl, F. and Caldwell, N. A Water-Soluble Benzodiazepine, RO 21-3981, for Induction of Anesthesia. *Anesthesiology, 49*: 41-43, 1978.

Fuxe, K. Agnati, F., Bolme, P., Hokfelt, T., Lidbrink, P., Ljungdahl, A., Perez de la Mora, M. and Ogren, S.-O. The Possible Involvement of GABA Mechanisms in the Action of Benzodiazepines on Central Catecholamine Neurons. In E. Costa and P. Greengard (Eds.), *Mechanism of Action of Benzodiazepines*. New York: Raven Press, 1975.

Gallager, D. W., Mallorga, P., Thomas, J. W. and Tallman, J. F. GABA-Benzodiazepine Interactions: Physiological, Pharmacological and Developmental Aspects. *Fed. Proc., 39*: 3043-3049, 1980.

Gale, G. and Galloon, S. Lorazepam as a Premedication. *Can. Anaesth. Soc. J., 23*: 22-29, 1976.

Gamble, J. A. S., Kawar, P., Dundee, J. W., Moore, J. and Briggs, L. P. Evaluation of Midazolam as an Intravenous Induction Agent. *Anaesthesia, 36*: 868-873, 1981.

Gilbert, B. W. and Hew E. M. Physiologic Significance of Hemodynamic Measurements and their Derived Indices. *Can. Med. Assoc. J., 121*: 871-876, 1979.

Gill, G. V., Prudhoe, K., Cook, D. B. and Latner, A. L. Effect of Surgical Trauma on Plasma Concentrations of Cyclic AMP and Cortisol. *Br. J. Surg., 62*: 441-443, 1975.

Glisson, S. N. Neurochemical Evicence for Cholinergic-Adrenergic Coupling in Mammalian Central Nervous Systems. Ph.D. Dissertation, Loyola University, Chicago, Illinois, 1971.

Glisson, S. N., Karczmar, A. G. and Barnes, C. Cholinergic Effects on Adrenergic Neuro Transmitters in Rabbit Brain Parts. *Neuropharmacology, 11*: 465-577, 1972.

Grandison, L. Anterior Pituitary GABA Receptors and their Regulation of Prolactin Secretion. In. E. Costa, G. Di Chiara and G. L. Gessa (Eds.), *GABA and Benzodiazepine Receptors*. New York: Raven Press, 1981.

Greenblatt, D. J. Clinical Pharmacokinetics of Oxazepam and Lorazepam. *Clin. Pharmacokinet., 6*: 89-105, 1981.

Greenblatt, D. J., Comer, W. H., Elliott, H. W., Shader, R. I., Knowles, J. A. and Ruelius, H. W. Clinical Pharmacokinetics of Lorazepam. III. Intravenous Injection. Preliminary Results. *J. Clin. Pharmacol., 17*: 490-494, 1977.

Guyton, A. C. *Textbook of Medical Physiology*. Philadelphia: W. B. Saunders Company, 1976.

Haber, E., Koerner, T., Page, L. B., Kimura, B., and Purnode, A. Application of Radioimmunoassay for Angiotensin I to the Physiologic Measurements of Plasma Renin Activity in Normal Human Subjects. *J. Clin. Endocrinol. Metab., 29*: 1349-1355, 1969.

Haefely, W. Kulcsar, A., Mohler, H., Pieri, L., Polc, P. and Schaffner, R. Possible Involvement of GABA in the Central Actions of Benzodiazepines. In E. Costa and P. Greengard (Eds.), *Mechanism of Action of Benzodiazepines*. New York: Raven Press, 1975.

Haggendal, J. An Improved Method for Fluorimetric Determination of Small Amounts of Adrenaline and Noradrenaline in Plasma and Tissues. *Acta Physiol. Scand., 59*: 242-254, 1963.

Hall, R.M., and Boulton, T.B. Editorial. *Anesthesia, 34*: 755-756, 1979.

Halter, J. B., Pflug, A. E. and Porte, D., Jr. Mechanism of Plasma Catecholamine Increases During Surgical Stress in Man. *J. Clin. Endocrinol. Metab., 45*: 936-944, 1977.

Hammond, W. G., Aronow, L. and Moore, F. D. Studies in Surgical Endocrinology. III. Plasma Concentrations of Epinephrine and Nor-epinephrine in Anesthesia, Trauma and Surgery, as Measured by a Modification of the Method of Weil-Malherbe and Bone. *Ann. Surg.*, *144*: 715-732 1956.

Hasbrouck, J. D. Morphine Anesthesia for Open-Heart Surgery. *Ann. Thorac. Surg.*, *10*: 364-369, 1970.

Hegarty, J. E. and Dundee, J. W. Sequelae After the Intravenous Injection of Three Benzodiazepines -- Diazepam, Lorazepam, and Flunitrazepam. *Br. Med. J.*, *2*: 1384-1385, 1977.

Heizmann, P. and Ziegler, W. H. Excretion and Metabolism of ^{14}C-Midazolam in Humans following Oral Dosing. *Arzneimittelforsch.*, *31* (*12a*): 2220-2223, 1981.

Helwig, J. T. and Council, K. A. (Eds.). *SAS User's Guide, 1979 Edition*. Cary, North Carolina: SAS Institute Inc., 1979.

Hickey, R.F., Eger, E.I. Circulatory Pharmacology of Inhaled Anesthetics. In R.D. Miller (Ed), *Anesthesia*. New York: Churchill Livingston, 1981.

Hillestad, L., Hansen, T. Melsom, H. and Drivenes, A. Diazepam Metabolism in Normal Man. *Clin. Pharmacol. Ther.*, *16*: 479-484, 1974.

Hine, I. P., Wood, W. G., Mainwaring-Burton, R. W., Butler, M. J., Irving, M. H. and Booker, B. The Adrenergic Response to Surgery Involving Cardiopulmonary Bypass, as Measured by Plasma and Urinary Catecholamine Concentratins. *Br. J. Anaesth.*, *48*: 355-363, 1976.

Hoar, P. F., Hickey, R. F. and Ullyot, D. J. Systemic Hypertension following Myocardial Revascularization. *J. Thorac. Cardiovasc. Surg.*, *71*: 859-864, 1976.

Hoar, P. F., Nelson, N. T., Mangano, D. T., Bainton, C. R. and Hickey, R. F. Adrenergic Responses to Morphine-Diazepam Anesthesia for Myocardial Revascularization. *Anesth. Analg.*, *60*: 406-411, 1981.

Hoar, P. F., Nelson, N. T., Mangano, D. T., Hickey, R. F. and Bainton, C. R. Adrenergic Response to Morphine and Valium Anesthesia. *Anesthesiology*, *53*: S105, 1980a.

Hoar, P.F., Stone, J.G., Faltas, A.N., Bendixen, H.H., Head, R.J. and Berkowitz, B.A. Hemodynamic and Adrenergic Responses to Anesthesia and Operation for Myocardial Revascularization. *J. Thorac. Cardiovasc. Surg., 80*: 242-248, 1980b.

Ikram, H., Rubin, A. P. and Jewkes, R. F. Effect of Diazepam on Myocardial Blood Flow of Patients with and without Coronary Artery Disease. *Br. Heart J., 35*: 626-630, 1973.

Iverson, L.L. and Salt, P.J. Inhibition of Catecholamine Uptake-2 by Steriods in the Isolated Rat Heart. *Br. J. Pharmacol., 40*: 528-530, 1970.

Innes, I.R. and Nickerson, M. Norepinephrine, Epinephrine, and the Sympathomimetic Amines. In L.S. Goodman and A. Gilman (Eds.), *The Pharmacological Basis of Therapeutics*. New York: Macmillan Publishing Co., Inc., 1975.

James, M. and Fisher, A. Nitrazepam as a Premedicant in Minor Surgery. *Anaesthesia, 25*: 364-367, 1970.

Jones, D. J., Stehling, L. C. and Zauder, H. L. Cardiovascular Responses to Diazepam and Midazolam Maleate in the Dog. *Anesthesiology, 51*: 430-434, 1979.

Kaneko, Y., Ikeda, T., Takeda, T. and Ueda, H. Renin Release During Acute Reduction of Arterial Pressure in Normotensive Subjects and Patients with Renovascular Hypertension. *J. Clin. Invest., 46*: 705-716, 1967.

Kaplan, J. A. Hemodynamic Monitoring. In J. A. Kaplan (Ed.), *Cardiac Anesthesia*. New York: Grune and Stratton, 1979.

Kaplan, J.A., Cardiovascular Physiology. In R.D. Miller (Ed), *Anesthesia*. New York: Churchill Livingston, 1981.

Kaplan, S. A., Jack, M. L., Alexander, K. and Weinfeld, R. E. Pharmacokinetic Profile of Diazepam in Man following Single Intravenous and Oral and Chronic Oral Administrations. *J. Pharm. Sci., 62*: 1789-1796, 1973.

Kehlet, H., Binder, C. and Engbaek, C. Cortisol Binding Capacity in Plasma During Anaesthesia and Surgery. *Acta Endocrinol.*, *75*: 119-125, 1974.

Khambatta, H. J., Stone, J. G. and Khan, E. Hypertension During Anesthesia on Discontinuation of Sodium Nitroprusside-Induced Hypotension. *Anesthesiology*, *51*: 127-130, 1979.

Kim, Y. D., Jones, M., Hanowell, S. T., Koch, J. P., Lees, D. E., Weise, V. and Kopin, I. J. Changes in Peripheral Vascular and Cardiac Sympathetic Activity Before and After Coronary Artery Bypass Surgery: Interrelationships with Hemodynamic Alterations. *Am. Heart J.*, *102*: 972-979, 1981.

Kirk, R. E. *Experimental Design: Procedures for the Behavioral Sciences*. Belmont, California: Brooks/Cole Publishing Company, 1968.

Klepner, C. A., Lippa, A. S., Benson, D. I., Sano, M. C. and Beer, B. Resolution of Two Biochemically and Pharmacologically Distinct Benzodiazepine Receptors. *Pharmacol. Biochem, Behav.*, *11*: 457-462, 1979.

Klotz, U., Antonin, K. H. and Bieck, P. R. Pharmacokinetics and Plasma Binding of Diazepam in Man, Dog, Rabbit, Guinea Pig and Rat. *J. Pharmacol. Exp. Ther.*, *199*: 67-73, 1976.

Knapp, R. B. and Fierro, L. Evaluation of the Cardiopulmonary Safety and Effects of Lorazepam as a Premedicant. *Anesth. Analg.*, *53:122-124*, *1974*.

Kothary, S. P., Zsigmond E. K. and Matsuki, A. Antagonism of the Ketamine Induced Rise in Plasma Free Norepinephrine, Blood Pressure and Pulse Rate by Intravenous Diazepam. *Clin. Pharmacol. Ther.*, *17*: 238, 1975.

Kumar, S. M., Kothary, S. P. and Zsigmond, E. K. Plasma Free Norepinephrine and Epinephrine Concentrations following Diazepam-Ketamine Anesthesia in Patients Undergoing Cardiac Surgery. *Acta Anaesthesiol. Scand.*, *22*: 593-600, 1978.

Kumar, S. M., Kothary, S. P. and Zsigmond, E. K. Lack of Cardiovascular Stimulation During Endotracheal Intubation in Cardiac Surgical Patients Anesthetized with Diazepam-Ketamine-Pancuronium. *Clin. Ther.*, *3*: 43-48, 1980.

Lappas, D. G., Fahmy, N. R., Moss, J. and Slater, E. E. Effects of Fentanyl-Diazepam Anesthesia on Hemodynamics, Plasma Catecholamines and Renin Activity in Critically-Ill Patients. *Anesthesiology*, *55*: A250, 1981.

Le Fur, G., Guilloux, F., Mitrani, N., Mizoule, J. and Uzan, A. Relationships between Plasma Corticosteroids and Benzodiazepines in Stress. *J. Pharmacol. Exp. Ther.*, *211*: 305-308, 1979.

Lewis, R. N. Plasma Hydrocortisone Concentrations in Relation to Anaesthesia and Surgery. *Br. J. Anaesth.*, *35*: 84-90, 1963.

Lidbrink, P. and Farnebo, L.-O. Uptake and Release of Noradrenaline in Rat Cerebral Cortex in Vitro: No effect of Benzodiazepines and Barbiturates. *Neuropharmacology*, *12*: 1087-1095, 1973.

Lippa, A. S., Critchett, D., Sano, M. C., Klepner, C. A., Greenblatt, E. N., Coupet, J. and Beer, B. Benzodiazepine Receptors: Cellular and Behavioral Characteristics. *Pharmacol. Biochem. Beh.*, *10*: 831-843, 1979.

Madsen, S. N., Brandt, M. R., Engquist, A., Badawi, I. and Kehlet, H. Inhibition of Plasma Cyclic AMP, Glucose and Cortisol Response to Surgery by Epidural Analgesia. *Br. J. Surg.*, *64*: 669-671, 1977.

Madsen, S. N., Engquist, A., Badawi, I. and Kehlet, H. Cyclic AMP, Glucose and Cortisol in Plasma During Surgery. *Horm. Metab. Res.*, *8*: 483-485, 1976.

Madsen, S. N., Fog-Moller, F., Christiansen, C., Vester-Andersen, T. and Engquist, A. Cyclic AMP, Adrenaline and Noradrenaline in Plasma During Surgery. *Br. J. Surg.*, *65*: 191-193, 1978.

Mandelli, M., Tognoni, G. and Garattini, S. Clinical Pharmacokinetics of Diazepam. *Clin. Pharmacokinet.*, *3*: 72-91, 1978.

Makara, G. B. and Stark E. Effect of Gamma-Aminobutyric Acid (GABA) and GABA Antagonist Drugs on ACTH Release. *Neuroendocrinology*, *16*: 178-190, 1974.

Markiewicz, W., Hunt, S., Harrison, D. C. and Alderman, E. L. Circulatory Effects of Diazepam in Heart Disease. *J. Clin. Pharmacol.*, *16*: *637-644*, *1976*.

Martin, I. L. and Candy, J. M. Facilitation of Specific Benzodiazepine Binding in Rat Brain Membrane Fragments by a Number of Anions. *Neuropharmacology*, *19*: 175-179, 1980.

McCammon, R. L., Hilgenberg, J. C. and Stoelting, R. K. Hemodynamic Effects of Diazepam and Diazepam-Nitrous Oxide in Patients with Coronary Artery Disease. *Anesth. Analg.*, *59*: 438-441, 1980.

McCann, S. M., Vijayan, E. and Negro-Vilar, A. Role of Gamma Aminobutyric Acid in Control of Anterior Pituitary Hormone Release. In E.Costa, G. Di Chiara and G. L. Gessa (Eds.), *GABA and Benzodiazepine Receptors*. New York: Raven Press, 1981.

McClish, A. Diazepam as an Intravenous Induction Agent for General Anaesthesia. *Can. Anaes. Soc. J.*, *13*: 562-575, 1966.

Melsom, M., Andreassen, P., Melsom, H., Hansen, T., Grendahl, H. and Hillestad, L, K. Diazepam in Acute Myocardial Infarction. Clinical Effects and Effects on Catecholamines, Free Fatty Acids and Cortisol. *Br. Heart J.*, *38*: 804-810, 1976.

Melvin, M. A., Johnson, B. H., Quasha, A. L. and Eger II, E. I. Induction of Anesthesia with Midazolam Decreases Halothane MAC in Man. *Anesthesiology*, *53*: S10, 1980.

Miller, E. D., Ackerly, J. A., Vaughan, E. D., Peach, M. J. and Epstein, R. M. The Renin-Angiotensin System During Controlled Hypotension with Sodium Nitroprusside. *Anesthesiology*, *47*: 257-252, 1977.

Mohler, H. and Okada, T. Benzodiazepine Receptors: Demonstration in the Central Nervous System. *Science*, *198*: 849-851, 1977.

Morel, D., Forster, A., Gardaz, J.-P., Suter, P. M. and Gemperle, M. Comparative Hemodynamic and Respiratory Effects of Midazolam and Flunitrazepam as Induction Agents in Cardiac Surgery. *Arzneimittelforsch.*, *31* (12a): 2264-2267, 1981.

Newsome, H. H. and Rose, J. C. The Response of Human Adrenocorticotrophic Hormone and Growth Hormone to Surgical Stress. *J. Clin. Endocrinol. Metab.*, *33*: 481-487, 1971.

Nickerson, M. and Ruedy, J. Antihypertensive Agents and the Drug Therapy of Hypertension. In L. S. Goodman and A. Gilman (Eds.), *The Pharmacological Basis of Therapeutics*. New York: Macmillan Publishing Co., Inc., 1975.

Nicol, C.J.M. and Rae, R.M. Inhibition of Accumulation of Adrenaline and Noradrenaline in Arterial Smooth Muscle by Steriods. *Br. J. Pharmacol.*, *44*: 361P-362P, 1972.

Nishi, S., Minota, S. and Karczmar, A. G. Primary Afferent Neurons: The Ionic Mechanim of GABA-Mediated Depolarization. *Neuropharmacology*, *13*: 215-219, 1974.

Oka, Y., Wakayama, S., Oyama, T., Orkin, L. R., Becker, R. M., Blaufox, M. D. and Frater, R. W. Cortisol and Antidiuretic Hormone Responses to Stress in Cardiac Surgical Patients. *Can. Anaesth. Soc. J.*, *28*: 334-339, 1981.

Oyama, T., Latto, P. and Holaday, D. A. Effect of Isoflurane Anaesthesia and Surgery on Carbohydrate Metabolism and Plasma Cortisol Levels in Man. *Can. Anaesth. Soc. J.*, *22*: 696-702, 1975.

Oyama, T., Taniguchi, K., Ishihara, H., Matsuki, A., Maeda, A., Murakawa, T. and Kudo, T. Effects of Enflurane Anaesthesia and Surgery on Endocrine Function in Man. *Br. J. Anaesth.*, *51*: 141-147, 1979.

Oyama, T., Taniguchi, K., Jin, T., Saton E. T. and Kudo, T. Effects of Anaesthesia and Surgery on Plasma Aldosterone Concentration and Renin Activity in Man. *Br. J. Anaesth.*, *51*: 747-751, 1979.

Palmer, R.F., and Lasseter, K.C. Sodium Nitroprusside. *N. Engl. J. Med. 292*: 294-297, 1975.

Paul, S. M., Marangos, P. J. and Skolnick, P. The Benzodiazepine-GABA-Chloride Ionophore Receptor Complex: Common Site of Minor Tranquilizer Action. *Biol. Psychiatry*, *16*: 213-229, 1981.

Perry, L. B., Van Dyke, R. A. and Theye, R. A. Sympathoadrenal and Hemodynamic Effects of Isoflurane, Halothane and Cyclopropane in Dogs. *Anesthesiology*, *40*: 465-470, 1974.

Pflug, A. E. and Halter, J. B. Effect of Spinal Anesthesia on Adrenergic Tone and the Neuroendocrine Responses to Surgical Stress in Humans. *Anesthesiology*, *55*: 120-126, 1981.

Philbin, D. M., Coggins, C. H., Emerson, C. W., Levine, F. H. and Buckley, M. Plasma Vasopressin Levels and Urinary Sodium Excretion During Cardiopulmonary Bypass. *J. Thor. Cardiovasc. Surg.*, *77*: 582-585, 1979.

Pieri, L., Schaffner, R., Scherschlicht, R., Polc, P., Sepinwall, J., Davidson, A., Mohler, H., Cumin, R., Da Prada, M., Burkard, W. P., Keller, H. H., Muller, R. K. M., Gerold, M., Pieri, M., Cook, L. and Haefely, W. Pharmacology of Midazolam. *Arzneimittelforsch.*, *31* (12a): 2180-2201, 1981.

Plumpton, F. S. and Besser, G. M. The Adrenocortical Response to Surgery and Insulin-Induced Hypoglycaemia in Corticosteriod-Treated and Normal Subjects. *Br. J. Surg.*, *56*: 216-219, 1969.

Racagni, G., Apud, J. A., Civati, C., Cocchi, D., Casanueva, F., Locatelli, V., Nistico, G. and Muller, E. E. Neurochemical Aspects of GABA and Glutamate in the Hypothalamo-Pituitary System. In E. Costa, G. Di Chiara and G. L. Gessa (Eds.), *GABA and Benzodiazepine Receptors*. New York: Raven Press, 1981.

Rawlinson, W. A., Loach, A. B., and Benedict, C. R. Changes in Plasma Concentration of Adrenaline and Noradrenaline in Anaesthetized Patients During Sodium Nitroprusside-Induced Hypotension. *Br. J. Anaesth.*, *50*: 937-943, 1978.

Reier, C. E., George, J. M. and Kilman, J. W. Cortisol and Growth Hormone Response to Surgical Stress During Morphine Anesthesia. *Anesth. Analg.*, *52*: 1003-1010, 1973.

Replogle, R., Levy, M., Dewall, R.A., and Lillehie, R.C. Catecholamine and Serotonin Response to Cardiopulmonary Bypass. *J. Thorac. Cardiocasc. Surg.*, *44*: 638-648, 1962.

Reves, J. G., Corssen, G. and Holcomb, C. Comparison of Two Benzodiazepines for Induction: Midazolam and Diazepam. *Can. Anaesth. Soc. J.*, *25*: 211-214, 1978.

Reves, J. G., Karp, R. B., Buttner, E. E., Tosone, S., Smith, L. R., Samuelson, P. N., Kreusch, G. R. and Oparil, S. Neuronal and Adrenomedullary Catecholamine Release in Response to Cardiopulmonary Bypass in Man. *Circulation*, *66*: 49-55, 1982.

Reves, J. G., Mardis, M. and Strong, S. Cardiopulmonary Effects of Midazolam. *Ala. J. Med. Sci.*, *15*: 347-351, 1978.

Reves, J. G., Samuelson, P. N. and Lewis, S. Midazolam Maleate Induction in Patients with Ischaemic Heart Disease: Haemodynamic Observations. *Can. Anaesth. Soc. J.*, *26*: 402-409, 1979.

Reves, J. G., Vinik, R., Hirschfield, A. M., Holcomb, C. and Strong, S. Midazolam Compared with Thiopentone as a Hypnotic Component in Balanced Anaesthesia: A Randomized, Double-Blind Study. *Can. Anaesth. Soc. J.*, *26*: 42-49, 1979.

Roberts, A. J., Niarchos, A. P., Subramanian, V. A., Abel, R. M., Herman, S. D., Sealey, J. E., Case, D. B., White, R. P., Johnson, G. A., Laragh, J. H. and Gay, W. A. Systemic Hypertension Associated with Coronary Artery Bypass Surgery. *J. Thorac. Cardiovasc. Surg.*, *74*: 846-859, 1977.

Roche Laboratories, *Nipride* package insert, August, 1982.

Roizen, M. F., Hamilton, W. K. and Sohn, Y. J. Treatment of Stress-Induced Increases in Pulmonary Capillary Wedge Pressure Using Volatile Anesthetics. *Anesthesiology*, *55*: 446-450, 1981.

Roizen, M. F., Moss, J., Henry, D. P. and Kopin, I. J. Effects of Halothane on Plasma Catecholamines. *Anesthesiology*, *41*: 432-439, 1974.

Rowe, G.G., and Henderson, R.H. Systemic and Coronary Hemodynamic Effects of Sodium Nitroprusside. *Am. Heart J.*, *87*: 83-87, 1974.

Samuelson, P. N., Lell, W. A., Kouchoukos, N. T., Strong, S. D. and Dole, K. M. Hemodynamics During Diazepam Induction of Anesthesia for Coronary Artery Bypass Grafting. *South. Med. J.*, *73*: 332-334, 1980.

Samuelson, P. N., Reves, J. G., Kouchoukos, N. T., Smith, L. R. and Dole, K. M. Hemodynamic Responses to Anesthetic Induction with Midazolam or Diazepam in Patients with Ischemic Heart Disease. *Anesth. Analg.*, *60*: 802-809, 1981.

Schwander, D. and Sansano, C. Cardiovascular Changes During Intubation with Midazolam as Anaesthesia Inducing Agent. *Arzneimittelforsch.*, *31* (12a): 2255-2260, 1981.

Sigg, E. B., Keim, K. L. and Kepner, K. Selective Effect of Diazepam on Certain Central Sympathetic Components. *Neuropharmacology*, *10*: 621-629, 1971.

Skultety, F. and Nishioka, H. The Results of Intracranial Surgery in the Treatment of Aneurysms. *J. Neurosurg.*, *25*: 683-704, 1966.

Smith, N. T., Eadie, M. J. and Brophy, T. O. The Pharmacokinetics of Midazolam in Man. *Eur. J. Clin. Phamacol.*, *19*: 271-278, 1981.

Smith, N.T., Calverley, R.K., Prys-Roberts, C., Eger, E.J.,II and Jones, C.W. Impact of Nitrous Oxide on the Circulation during Enflurane Anesthesia in Man. *Anesthesiology*, *48*: 345-349, 1978.

Speth, R. C., Johnson, R. W., Regan, J., Reisine, T., Kobayash, B., Resolin, N., Roeske, W. R. and Yamamura, H. I. The Benzodiazepine Receptor of Mammalian Brain. *Fed. Proc.*, *39*: 3032-3038, 1980.

Squires, F. R., Benson, D. I., Braestrup, C., Coupet, J., Klepner, C. A., Myers, V., Beer,B. Some Properties of Brain Specific Benzodiazepine Receptors: New Evidence. *Pharmacol. Biochem. Beh.*, *10*: 825-830, 1979.

Squires, R. F. and Braestrup, C. Benzodiazepine Receptors in Rat Brain. *Nature*, *266*: 732-734, 1977.

Stanek, B., Zimpfer, M., Fitzal, S. and Raberger, G. Plasma Catecholamines, Plasma Renin Activity and Haemodynamics During Sodium Nitroprusside-Induced Hypotension and Additional Beta-Blockade with Bunitrolol. *Eur. J. Clin. Pharmacol.*, *19*: 317-322, 1981.

Swales, J. D. Renin-Angiotensin System in Hypertension. *Pharmac. Ther.*, *7*: 173-201, 1979.

Sweet, C. S. Pharmacological Aspects of the Renin-Angiotensin System. In M. J. Antonaccio (Ed.), *Cardiovascular Pharmacology*. New York: Raven Press, 1977.

Sweet, C. S., Wenger, H. C. and Gross, D. M. Central Antihypertensive Properties of Muscimol and Related Gamma-aminobutyric Acid Agonists and the Interaction of Muscimol with Baroreceptor Reflexes. *Can. J. Physiol. Pharmacol.*, *57*: 600-605, 1979.

Tallman, J. F., Thomas, J. W. and Gallager, D. W. GABAergic Modulation of Benzodiazepine Binding Site Sensitivity. *Nature*, *274*: 383-385, 1978.

Tan, C., Glisson, S. N., El-Etr, A. A. and Younes, S. H. Adrenal Responses to Anesthetics During Cardiopulmonary Bypass. *Cardiovasc. Med. 3*: 521-528, 1978.

Tappaz, M. L., Aguera, M., Belin, M. F., Oertel, W. H., Schmechel, D. E., Kopin, I. J. and Pujol, J. F. GABA Markers in the Hypothalamic Median Eminence. In E. Costa, G. Di Chiara and G. L. Gessa (Eds.), *GABA and Benzodiazepine Receptors*. New York: Raven Press, 1981.

Taylor, K. M., Jones, J. V., Walker, M. S., Rao, S. and Bain, W. The Cortisol Response During Heart-Lung Bypass. *Circulation*, *54*: 20-25, 1976.

Taylor, K. M. and Laverty, R. The Interaction of Chlordiazepoxide, Diazepam, and Nitrazepam with Catecholamines and Histamine in Regions of the Rat Brain. In S. Garattini, E. Mussini and L. O. Randall (Eds.), *The Benzodiazepines*. New York: Raven Press, 1973.

Taylor, K. M., Morton, I. J., Brown, J. J., Bain, W. H. and Caves, P. K. Hypertension and the Renin-Angiotensin System following Open-Heart Surgery. *J. Thor. Cardiovasc. Surg.*, *74*: 840-845, 1977.

Turndorf, H. Discussion of C. E. Reier, J. M. George, J. W. Kilman, Cortisol and Growth Hormone Response to Surgical Stress During Morphine Anesthesia. *Anesth. Analg.*, *52*: 1009-1010, 1973

Turton, M. B., Deegan, T. and Coulshed, N. Plasma Catecholamine Levels and Cardiac Rhythm Before and After Cardiac Catheterization. *Br. Heart J.*, *39*: 1307-1311, 1977.

Van Ackern, K., Franke, N., Peter, K. and Schmucker, P. Enflurane in Patients with Coronary Artery Disease. *Acta Anaesthesiol Scand.* (Suppl.), *71*: 71-76, 1979.

Vendsalu, A. Studies on Adrenalin and Noradrenalin in Human Plasma. *Acta Physiol. Scand.* (*Suppl. 173*), *49*: 1-123, 1960

Viljoen, J. F., Estafanous, F. G. and Tarazi, R. C. Acute Hypertension Immediately After Coronary Artery Surgery. *J. Thorac. Cardiovasc. Surg.*, *71*: 548-550, 1976.

Vlachakis, N. D., Pratilas, V. and Pratila, M. Raised Plasma Catecholamines. *N. Y. State J. Med.*, *81*: 27-35, 1981.

Vree, T. B., Baars, A. M., Booij, L. H. D. and Driessen, J. J. Simultaneous Determination and Pharmacokinetics of Midazolam and its Hydroxymetabolites in Plasma and Urine of Man and Dog by Means of High-Performance Liquid Chromatography. *Arzneimittelforsch.*, *31* (12a): 2215-2219, 1981.

Wallenstein, S. Zucker, C. L. and Fleiss, J. L. Some Statistical Methods Useful in Circulation Research. *Circ. Res.*, *47*: 1-9, 1980.

Wesseling, H. and Edens, E. T. Effect of Premedication on Stress and Plasma Cortisol in Patients Bronchoscoped under Local Anaesthesia. *Eur. J. Clin. Pharmacol.*, *8*: 323-326, 1975.

White, R. D. Monitoring Arrhythmias and Signs of Intraoperative Ischemia by Electrocardiogram. *Cleve. Clin. Q.*, *48*: 32-36, 1981.

Wilson, J. Lorazepam as a Premedicant for General Anaesthesia. *Curr. Med. Res. Opin.*, *1*: 308-316, 1973.

Winer, B. J. *Statistical Principles in Experimental Design*. New York: McGraw-Hill Book Company, 1962.

Yogo, H., Sasaki, T., Yamaoka, T., Naruse, T. and Itasaka, Y. Response of Plasma Renin Activity During Surgical Stress. *Jpn. J. Surg.*, *3*: 203-211, 1973.

Yokota, H., Kawashima, Y., Hashimoto, S., Manabe, H., Onishi, T., Aono, T. and Matsumoto, K. Plasma Cortisol, Luteinizing Hormone (LH), and Prolactin Secretory Responses to Cardiopulmonary Bypass. *J. Surg. Res.*, *23*: 196-200, 1977.

Yun, J. C., Donahue, J. J., Bartter, F. C. and Kelly, G. D. Effect of Pentobarbital Anesthesia and Laparotomy on Plasma Renin Activity in the Dog. *Can. J. Physiol. Pharmacol.*, *57*: 412-416, 1979.

Zivin, J. A. and Bartko, J. J. Statistics for Disinterested Scientists. *Life Sci.*, *18*: 15-26, 1976.

Zsigmond, E. K., Kothary, S. P., Kumar, S. M. and Kelsch, R. C. Counteraction of Circulatory Side Effects of Ketamine by Pretreatment with Diazepam. *Clin. Ther.*, *3*: 28-32, 1980.

Zsigmond, E. K., Kothary, S. P., Matsuki, A. and Kelsch, R. C. Diazepam for Prevention of the Rise in Plasma Catecholamines Caused by Ketamine. *Clin. Pharmacol. Ther.*, *15*: 223-224, 1974.

APPENDIX A

TABLE 8

ANALYSIS OF VARIANCE FROM EXPERIMENT I

	F-Ratio		
	Drug	Time	Drug*Time
Blood Pressure	0.5	328.6*	1.0
Heart Rate	0.3	2.6*	0.6
Cardiac Index	2.2	12.7*	1.3
Right Atrial Pressure	0.3	3.8*	1.0
Systemic Vascular Resistance Index	1.8	27.8*	0.8
Wedge Pressure	1.2	2.7*	1.0
Stroke Work Index	2.2	80.5*	0.9
Epinephrine	7.2*	4.3*	1.7
Norepinephrine	6.0*	0.5	2.2*
Cortisol	2.2	10.4*	1.5
Renin Activity	0.6	24.3*	1.1

*p less than .05

TABLE 9

ANALYSIS OF VARIANCE FROM EXPERIMENT II

	F-Ratio		
	Drug	Time	Drug*Time
Blood Pressure	1.6	119.0*	0.3
Heart Rate	0.8	3.7*	0.4
Cardiac Index	7.1*	12.9*	0.6
Right Atrial Pressure	1.6	6.0*	1.7
Systemic Vascular Resistance Index	7.2*	15.7*	0.8
Wedge Pressure	2.9	7.3*	3.5*
Stroke Work Index	0.3	65.9*	0.9
Epinephrine	7.0*	4.8*	1.5
Norepinephrine	4.8*	2.1	0.8
Cortisol	0.8	15.7*	2.9*

*p less than .05

TABLE 10

ANALYSIS OF VARIANCE FROM EXPERIMENT III

	F-Ratio		
	Drug	Time	Drug*Time
Blood Pressure	0.1	14.6*	1.0
Heart Rate	3.2	6.3*	0.2
Cardiac Index	1.2	6.4*	0.2
Right Atrial Pressure	0.1	1.3	2.0
Systemic Vascular Resistance Index	1.5	1.4	0.5
Wedge Pressure	0.0	3.1*	0.5
Stroke Work Index	2.3	11.3*	0.4
Epinephrine	9.0*	0.8	1.0
Norepinephrine	3.2	0.5	1.0
Cortisol	0.3	3.7*	1.0
Renin Activity	2.8	8.3*	3.2*

*p less than .05

TABLE 11

ANALYSIS OF VARIANCE
FROM POSTINCISION BLOOD PRESSURE AND HEART RATE

	F-Ratio		
	Drug	Time	Drug*Time
Blood Pressure	0.7	2.7*	5.0*
Heart Rate	1.4	3.7*	1.7

*p less than .05

APPENDIX B

TABLE 12

TYPICAL PLASMA EPINEPHRINE STANDARD CURVE DATA

Sample Epinephrine Concentration (ng/ml)	NET FLUOROMETRIC UNITS	
	400/505 nM	450/505 nM
1	9	3
2.5	24	8
5	45	16
10	95	36
15	147	57
20	195	77
25	249	95
50	507	195

TABLE 13

TYPICAL PLASMA NOREPINEPHRINE STANDARD CURVE DATA

Sample Norepinephrine Concentration (ng/ml)	NET FLUOROMETRIC UNITS	
	400/505 nM	450/505 nM
1	4	1
2.5	9	1
5	20	2
10	48	5
15	75	7
20	100	10
25	127	12
50	263	13

TABLE 14

TYPICAL PLASMA CORTISOL STANDARD CURVE DATA

Sample Cortisol Concentration (mcg/dl)	%B/Bo[1]
1.0	80.2
1.0	80.3
4.3	60.0
4.3	59.3
10.6	42.2
10.6	41.9
28.6	25.2
28.6	25.9
60.9	17.4
60.9	17.2

[1] B = Sample Counts
Bo = Average Zero Standard Counts

TABLE 15

TYPICAL PLASMA RENIN ACTIVITY STARDARD CURVE DATA

Sample Angiotensin I (ng/tube)	%B/Bo[1]
.020	74.7
.020	71.6
.040	56.0
.040	60.4
.080	39.6
.080	38.1
.125	30.0
.125	28.6
.250	16.1
.250	15.7
.500	8.1
.500	9.1

[1] B = Sample Counts
Bo = Average Zero Standard Counts

APPROVAL SHEET

The dissertation submitted by Gail Elizabeth Gillenwater has been read and approved by the following committee:

Dr. Silas N. Glisson, Director
Associate Professor, Anesthesiology & Pharmacology, Loyola

Dr. Alexander G. Karczmar
Professor and Chairman, Pharmacology
Associate Dean, Graduate School, Loyola

Dr. Adel A. El-Etr
Professor and Chairman, Anesthesiology, Loyola

Dr. John Z. Zaroslinski
Adjunct Professor, Pharmacology, Loyola

Dr. Stephen B. Jones
Associate Professor, Physiology, Loyola

The final copies have been examined by the director of the dissertation and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the dissertation is now given final approval by the Committee with reference to content and form.

The dissertation is therefore accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

April 17, 1984
Date

Silas N. Glisson
Silas N. Glisson, Ph.D.
Director