INFLUENCE OF PENTOBARBITAL AND MORPHINE-CHLORALOSE ANESTHESIA ON THE AUTONOMIC CARDIOVASCULAR FUNCTION AND ON DRUG RESPONSES IN MONGREL DOGS

A Thesis

Presented to

the Faculty of the College of Pharmacy

University of Houston

In Partial Fulfillment

of the Requirements for the Degree

Master of Science in Pharmacy

---

by

Amin Tawfig Hamed

August, 1978

"DEDICATED TO MY PARENTS, WHOSE FLOW

OF LOVE NEVER CEASED TO INSPIRE ME"

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ABSTRACT

The major objectives of this investigation were to evaluate certain autonomic and cardiovascular effects of morphine-chloralose and pentobarbital anesthesia in mongrel dogs and to determine the influence of the two anesthetics on the effect of certain pharmacological agents.

Alterations in the hindleg vascular reactivity and cardiovascular reflexes produced by pentobarbital (P) 35 mg/kg, i.v., and morphine (M) 3 mg/kg, i.m., plus chloralose (C) 100 mg/kg, i.v., were evaluated in mongrel dogs. Comparison of pressure-flow curves indicated that there was a significant reduction in the hind limb vascular resistance in dogs anesthetized with P. However, acute denervation plus the administration of an alpha-blocker produced identical shifts of the pressure-flow curves to the right in both groups, suggesting that the neurogenic tone and tone contributed by circulating catecholamines were of equal magnitude in the dogs under the influence of P or M + C. Thus, the major difference appears to be that the intrinsic vascular tone of the smooth muscle was significantly depressed by P anesthesia.

Reflex increase and decrease in perfusion pressure in response to bilateral carotid occlusion and i.v. norepinephrine respectively, were significantly greater in M + C dogs. Reflex bradycardic responses to i.v. phenylephrine and norepinephrine were also significantly greater in M + C animals compared to those anesthetized with P. Further, blood pressure and heart rate responses to carotid sinus nerve stimulation were more pronounced in the M + C animals. These results indicate that while the resting neurogenic tone to the hind limb vasculature is

identical in M + C or P anesthetized dogs, cardiovascular reflexes are more responsive in M + C anesthetized animals.

Administration of clonidine (20 μg/kg, i.v.) caused a potentiation in the reflex bradycardic response to norepinephrine in the P anesthetized animals when compared with the M + C dogs and significantly potentiated the bradycardia in response to phenylephrine in both groups of anesthetized dogs. Δ^9-tetrahydrocannabinol (Δ^9-THC) administration, 2.5 mg/kg, i.v., to M + C anesthetized animals caused an increase in heart rate and an increase in the pulmonary blood flow and a decrease in blood pressure, total peripheral resistance, pulmonary artery pressure and pulmonary vascular resistance. Δ^9-THC produced opposite effects to those mentioned above in conscious and P anesthetized dogs.

Evaluating the effect of M + C, P and SQ-14,225 (an angiotensin converting enzyme inhibitor) on the plasma renin activity (PRA) of mongrel dogs showed that both M + C and P caused a significant increase in the PRA at 15, 30 and 60 minutes after administration. However, the increase in the PRA was twice as great in the M + C dogs at 30 and 60 minutes as it was in those anesthetized with P. SQ-14,225 caused a significant increase in the PRA of both groups of animals at 15,30 and 60 minutes after its administration. Further, SQ-14,225 caused a similar decrease in blood pressure in both groups, accompanied by a significant increase in heart rate at 15 minutes that was followed by partial recovery in the M + C animals. Dogs anesthetized with P showed a slight decrease in heart rate. The present study provides evidence for the postulation that the quantity and quality of responses produced by pharmacological agents depend upon whether the animals are anesthetized or not, and if so, the type of anesthesia used.

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I. Introduction and Literature Survey

Anesthetic agents such as pentobarbital, urethane, alpha-chloralose and morphine are the most widely used in various experimental studies. In spite of the amount of work that has been done on the effect of these and other anesthetics on the central nervous system, the autonomic and cardiovascular systems, a great deal of controversy still surrounds how the use of these agents change the so-called normal animal into a physiologically abnormal one.

These commonly used agents possess various physiological and pharmacological properties that should be taken into consideration when drug actions and mechanisms are investigated in animals which are under the influence of one or more of the anesthetics. The current studies are designed to investigate the cardiovascular and autonomic effects of the two most commonly used anesthetics, or their combinations, so as to obtain information on how these agents may influence drug action.

1. Anesthetic's Mechanism of Action

The ability of anesthetics to produce unconsciousness, and the physical-chemical changes that take place in the central nervous system are still obscure and not fully understood. However, several mechanisms of action have been proposed.

A. Larrabbee and Pasternak (1), after they compared impulses transmitted synaptically and nonsynaptically, proposed that most anesthetics produce unconsciousness by preferential blockade of an asynaptic process. This has led to the suggestion that anesthetics act, at least in part, by inhibition of multisynaptic pathways in the central nervous

system. The effect on multisynaptic pathways might be produced in several ways. Sensitivity of individual neurons to transmitter substances can be markedly changed by anesthetic agents (2). The release of transmitter substances may be inhibited by anesthetics (3). Alteration of the threshold potential which induces neuronal discharge has also been observed during anesthesia (4).

B. Molecular theories: These are based on the widespread interaction of the inert anesthetic gases with all components of the cell, namely: lipid, protein, and perhaps, water (5,6). Mullins (6) speculated that occlusion of a certain volume of free space in a cell membrane by the presence of anesthetic molecules might lead to an inability of sodium ions to pass through the membrane, which would subsequently inhibit neuronal discharge by preventing that selective increase in sodium permeability that takes place during excitation.

C. Biochemical effects: Kalow (7) and Quastel (8) suggested that the ability of drugs to inhibit oxidative phosphorylation or to decrease the available energy for the process of maintaining neural function is related to their ability to produce anesthesia. Bunker and Vandam (9) and Fink et al., (10) believe that the changes observed in energy transfer systems of the brain during inhalation anesthesia are probably secondary rather than primary processes. These and other factors may play a role in the ability of agents to produce anesthesia, but the means by which anesthetics affect metabolism are not completely understood at the present time.

2. Anesthetics on Centrally Mediated Actions

It is often assumed that the circulatory effects of general anesthetics result from direct action exerted on the heart or on the medullary vasomotor center. However, it is also likely that reflex responsiveness depends greatly upon the interplay of inhibitory and facilatory impulses which reach the medulla from higher centers such as the hypothalamus, diencephalon and the cortex.

Sodium pentobarbital, when injected into the lateral ventricles of chloralose anesthetized cats, was found to prevent cardiac arrhythmias which ordinarily occurred when caffeine was introduced into the same area. This action was attributed to depression of hypothalamic nuclei by the anesthetic. Intravenous injection of this barbiturate (200 mg) prevented the appearance of ventricular extrasystole which otherwise occurred when the carotid arteries were occluded (11).

Hypothalamic areas controlling the force of cardiac contraction were postulated in conscious, vagotomized cats by Manning and Peiss (12). Forel's field, zona incerta, hypothalamus lateralis and median forebrain bundle all contained areas which, upon electrical stimulation, caused large increases in pulse pressure unaccompanied by comparable alterations in diastolic blood pressure or heart rate. Hypnotic doses of pentobarbital greatly elevated the threshold to stimulation in this area, but hardly affected the response of the medullary vasomotor center (12).

Hypothalamic modification of pressor reflex was shown by Redgate and Gellhorn (13). In cats anesthetized with chloralose, the injection

of several milligrams of thiopental or pentobarbital into the posterior hypothalamus produced arterial hypotension and bradycardia in all cases. These injections also diminished the reflex increase in sympathetic neuronal activity accompanying hypotension. Introduction of the same drugs into the anterior hypothalamus resulted in inconsistent elevation of blood pressure and heart rate, but the reflex bradycardia provoked by norepinephrine injection was reduced and the pressor response conspicuously increased (14). These findings suggest an atonic effect of the hypothalamus upon the regulation of blood pressure, heart rate and cardiac output. The difference between the effects of anterior and posterior hypothalamic injection suggests that intravenous administration of a barbiturate could result either in increased or decreased arterial pressure and tachycardia or bradycardia, depending upon which area was dominant.

Anesthetics such as cyclopropane, and in particular, chloroform, were found to cause stimulation of the posterior hypothalamus in the dog (15,16,17), resulting in an increased discharge from the adrenal medulla and the cardiac sympathetic nerves. Abnormal cardiac rhythms during chloroform anesthesia apparently resulted from this increase in sympathetic discharge. Hypothalamic stimulation by cyclopropane was thought to result reflexly from actions exerted by the anesthetic on a postulated receptor located in the mesentery (18). Diethylene was found to be less of a depressant than amytal on the posterior hypothalamus (19).

Electrical stimulation of the anterior cerebral cortex of the dogs anesthetized with pentobarbital (25 mg/kg) produced arterial

hypotension, while under diethyl ether anesthesia the response was usually pressor (20).

3. Effect of Anesthetics on the Cardiovascular and Autonomic Nervous System

Nash et al., (21) studied the cardiovascular effects of anesthetic doses of pentobarbital sodium in dogs over a period of seven hours. There was a sudden but marked increase in heart rate from a control value of 93 beats/min to 164 beats/min about one-half hour after the induction dose had been given. This marked increase in heart rate was presumed to be due to the vagal inhibition by pentobarbital as shown by Kippanyi (22) and Linegar et al., (23). Following the administration of pentobarbital, cardiac output fell progressively during the first 2-3 hours; the maximum fall exceeding 44% of control value. The total peripheral resistance increased as the cardiac output decreased. This rise in resistance was inversally proportional to the decrease in cardiac output. Left ventricular work decreased with cardiac output. These investigators also reported that except for the brief period during induction of the anesthesia, the mean blood pressure did not deviate markedly from that of the unanesthetized animals. The fall in hematocrit during the first hour was followed by a slow partial recovery. Plasma proteins decreased both in concentration and in absolute amount, while plasma volume showed a slight rise (22). The authors concluded that the widely used anesthetic dose of pentobarbital (30 mg/kg) seems needlessly high, and may be expected to induce cardiovascular changes even greater than those reported here. While the mechanism of action

for the large fall in cardiac output has not been elucidated, it appears likely that a direct action on the myocardium may be involved. This conclusion was supported by the work of Roth (24) who perfused the isolated rat heart with 0.05 M pentobarbital and found a depression of short duration both in rate and amplitude of contraction. Also, it was supported by Johnson (25) who perfused the turtle heart with .002 M pentobarbital and found a pronounced reduction in the amplitude of contraction.

In 1950, Morrison et al., (26) investigated the effect of pentobarbital on the response of the cardiovascular system of dogs to epinephrine and acetylcholine. The investigators reported that even with the smallest dose of pentobarbital (2.5 mg/kg) there was a gradual fall in mean blood pressure. This was associated with a moderate increase in pulse rate. As the dose was increased these effects became more marked. The increase in blood pressure in response to the administered epinephrine under pentobarbital was larger and the bradycardiac response was less pronounced under the anesthesia. The increased pressor response to epinephrine appeared to be closely related to a disappearance of reflex bradycardia; in deep anesthesia, epinephrine occasionally produced a tachycardia rather than a bradycardia. These effects are explained on the basis of depression of those reflexes which tend to counteract rise in pressure.

Animals under the influence of pentobarbital are increasingly sensitive to acetylcholine. These changes were most evident in a prolongation of the time during which the depressor effect lasted.

The increased sensitivity to acetylcholine was closely correlated with a disappearance of reflex tachycardia.

Daniel et al., (27) analyzed the mechanism of barbiturate induced cardiovascular depression and its antagonism by sympathomimetic amines. These authors concluded that cardiovascular failure in dogs during continuous barbiturate infusions follows a characteristic pattern. The onset of hypotension occurs soon after the beginning of pentobarbital infusion and the blood pressure falls steadily until collapse is complete. Pentobarbital and thiopental depress the myocardium, decreasing cardiac index, cardiac output and raising right atrial pressure. The development of cardiovascular depression during barbiturate infusion was delayed by simultaneous infusion of norepinephrine. Heart failure induced by barbiturates is primarily the result of their negative inotropic action. However, suppression of autonomic rhythmicity and conduction is responsible for cardiac arrest when contractility is preserved by norepinephrine infusion, and these arrests occur when excitability is relatively unimpaired.

Barlow and Knott (28) studied the hemodynamic alterations after 30 minutes of pentobarbital anesthesia in dogs. Pentobarbital (30 mg/kg) caused increase in mean arterial pressure and cardiac output which is contradictory to what Nash (21) and Daniel (27) had reported. The increase in blood pressure is in agreement with what Corcoran (29) had reported. Further, Barlow reported a marked increase in heart rate and peripheral resistance in the anesthetized dogs. These workers also showed a significant decrease in blood volume. Barlow and Knott (28)

concluded that pentobarbital sodium exerts a profound influence on the cardiovascular system, reminding that the ubiquity of pentobarbital anesthesia in experimental medicine mandates that the investigator appreciate the altered cardiovascular system with which he is working. They also added that blood flow, blood pressure and resistance to flow are very markedly influenced by pentobarbital. Also in 1964, Peiss and Manning (30) studied the effects of sodium pentobarbital on electrical and reflex activation of the cardiovascular system in dogs. In the dose of 5 mg/kg, i.v., pentobarbital caused a reduction in both systolic and diastolic pressure in response to hypothalamic stimulation; this was in agreement with what Masserman (19) had previously shown. The cardiac responses to sympathetic stimulation of the hypothalamus in the cat are markedly decreased by sodium amytal in 20 to 50 mg/kg doses. Masserman also demonstrated a decrease in blood pressure when small amounts of this drug were injected directly into the hypothalamus. Further, Redgate and Gellhorne (13) reported that injection of sodium thiopental into the posterior hypothalamus of cats produced a fall in blood pressure and heart rate.

Peiss (30) also showed a moderate depression of cardiovascular responses to stimulation of the dorsal medullary reticular formation after administration of pentobarbital. Furthermore, these investigators reported a marked depression of the carotid sinus response in heart rate, pulse pressure, and diastolic pressure after pentobarbital. This was in agreement with the reports of Bouckaert and Heymans (31).

In 1965, Gilmore (32) studied the effect of pentobarbital on the circulation of the dog. The data indicated that after initial transient

hypotension, pentobarbital sodium had little influence on the mean arterial pressure of normotensive dogs, which confirmed the work of Nash (21) and disagreed with that of Daniel (27), Corcoran (29) and Barlow et al., (28). Cardiac output was unchanged one hour following anesthesia, then decreased approximately 25% and remained at this level up to 4 hours after anesthesia. However, Nash (21) had reported a decrease in cardiac output of 44% by the third hour.

It was also shown that estimated hepatic blood flow is not modified by pentobarbital sodium, but the splanchnic A-VO_2 difference decreases. Pentobarbital appears to produce hemodilution from the splenic sequestration of erythrocyte, and within 30 minutes after the injection of pentobarbital anesthesia to normal dogs, hematocrit, leukocyte count and rectal temperature had decreased. These experiments confirmed the work of Nash et al., (21) who had obtained similar results.

Cox (33) investigated the influence of pentobarbital anesthesia on cardiovascular function in trained dogs. The data indicated that with the exception of a significantly increased heart rate and decreased stroke volume, there were no significant changes in the hemodynamic variables due to the induction of the pentobarbital anesthesia. These variables include mean arterial pressure, cardiac output, total peripheral resistance, left ventricular pressure and its first derivative (dp/dt), cardiac index, left ventricular diastolic volume and venous pressure. It should be noted that Cox studied these "trained" dogs only for 60 minutes, which was quite to the contrary to Barlow (28), who reported a marked increase in mean arterial pressure, cardiac output and peripheral resistance after only 30 minutes of pentobarbital induction,

and to Nash (21) who reported a slight decrease in mean blood pressure, cardiac output, cardiac index and a slight increase in total peripheral resistance after 60 minutes of pentobarbital injection. The hemodynamic responses reported by Cox were found to be dependent upon the level of vagal tone. It has been shown by Koppanyi (22) and by Linegar et al., (23) that pentobarbital inhibits vagal tone to the dog heart. However, Manders and Vatner (34) showed the increase in the heart rate is not only due to inhibition of vagal tone, but it is predominantly mediated through the arterial baroreceptor reflex.

Lokhandwala et al., (35) demonstrated that pentobarbital anesthesia caused inhibition of vagal tone with subsequent enhancement of sympathetic tone to the myocardium. Manders and Vatner (34) also showed that pentobarbital anesthesia attenuated the effect of isoproterenol on heart rate, cardiac output and blood pressure, indicating that this anesthetic depresses the S-A node and responsiveness of the vascular smooth muscle.

Forsyth and Hoffbrand (36) investigated the redistribution of cardiac output after sodium pentobarbital anesthesia in the monkey. After anesthesia (30 mg/kg), higher percentages of cardiac output were delivered to the kidneys, skin, lungs (bronchial artery) and bone at the expense of brain, skeletal muscle, adrenal and chest wall. These latter organs received significantly decreased blood flow. Only the lungs had a significantly higher flow. The brain was the only organ to have significantly higher resistance.

Johnson and Malvin (37) studied the plasma renin activity during pentobarbital anesthesia in dogs. The investigators reported a fivefold increase in plasma renin activity within 15 minutes, followed by a slow decline to a level 3 times control at 1.5 hours. Lokhandwala et al., (38) also reported that pentobarbital anesthesia produced a fourfold increase in plasma renin activity in mongrel dogs.

It is believed that many of the effects produced by pentobarbital are in one way or another related to calcium (Ca^{+2}). Winifred and Szeto (39) reported that pentobarbital alters the cellular distribution of Ca^{+2} and quantity of bound Ca^{+2} available for displacement of Lanthanum (La^{+3}). Dransfeld et al., (40) reported that pentobarbital inhibits Ca^{+2} uptake by sacroplasmic reticulum isolated from heart muscle as the microsomal fraction and incubated in a reaction mixture containing oxalate. In addition, they showed that it uncouples Ca^{+2} transport and ATP hydrolysis in 1, 2 and 3 mM concentrations of pentobarbital. Hess et al., (41), have shown that barbiturates interact with phospholipids prepared from isolated fragments of sacroplasmic reticulum, thereby altering the ability of these phospholipids to bind Ca^{+2}.

In recent years, it has been shown that alpha-chloralose anesthesia might have advantages in some types of investigations. Citters et al., (42) recommended alpha-chloralose in studies on the central neuronal regulation of the cardiovascular system since reflex activity is better maintained. Furthermore, alpha-chloralose had less effect on basal cardiac performance than pentobarbital anesthesia. In 1971 Arfors et al., (43) investigated the effect of prolonged chloralose

anesthesia on acid-base balance and cardiovascular function in dogs. After induction of chloralose (in polyethylene glycol) anesthesia, the mean value of pulmonary artery PCO_2 was slightly higher than that of conscious dogs but the difference was not significant. The arterial pH tended to decrease but not significantly. The mean arterial pressure was higher than in the conscious group of dogs one hour after the induction of anesthesia and remained relatively stable. The heart rate increased moderately during anesthesia; there were no significant changes in cardiac index or O_2 consumption observed during anesthesia. Mean values of cardiac output were essentially unchanged.

Cox (44) evaluated the influence of chloralose anesthesia on cardiovascular function in trained dogs. The induction of anesthesia with chloralose (100 mg/kg) was associated with substantial transient hemodynamic changes which lasted about 5-15 minutes after the initiation of administration. The principal transient effects were a peripheral vasodilation, a loss of arrhythmia and resulting tachycardia, myocardial depression and central venous pooling. No significant differences were observed in response to norepinephrine doses, while the heart period and stroke volume were higher after chloralose anesthesia. Isoproterenol responses were virtually unaltered by chloralose anesthesia, but were depressed by pentobarbital anesthesia (33).

The only variable affected by chloralose anesthesia are the left ventricular pressure derivative and the aortic flow derivative, both of which were increased. These results could be interpreted to indicate that chloralose anesthesia enhances myocardial contractile force.

However, just the opposite have been reported by Price (45), who showed that chloralose causes direct myocardial depression, but sympathetic outflow to the heart could increase as a compensatory mechanism to depression, and chloralose has been reported to increase the excitability of the sympathetic nervous system (46). Therefore, the increase in these indices is possibly due to the increase in sympathetic nerve activity.

Intact dogs, maintained at constant depth of anesthesia with intravenous chloralose infusion exhibited a progressively rising heart rate which stabilized at an elevated level after 1-1/2 hours. Stroke volume was stable for 3 hours and then steadily declined, and the arterial pressure remained at a higher level throughout the experimental period (47).

Bass and Buckley (47) also reported a marked depression of function in the heart lung preparation; there was a decrease in stroke volume, left ventricular stroke work and ventricular dp/dt, and a rise in ventricular end diastolic pressure when alpha-chloralose was added to the preparation.

Ngai and Bolme (48) investigated the effects of cyclopropane, halothane, enibomal sodium (narcobarbital) and morphine on circulatory regulating mechanisms in dogs. During light cyclopropane anesthesia, the arterial pressure rose and vascular resistance in the hind leg increased. As anesthesia was deepened, the heart rate decreased and changes in arterial pressure were inconsistent. With halothane anesthesia, there was bradycardia and progressive hypotension. After administration of enibomal, tachycardia and hypotension occurred.

Sedation with morphine was accompanied by bradycardia but no significant change in arterial pressure. Cyclopropane, halothane and enibomal depressed the pressor response to central stimulation and carotid occlusion. The authors concluded that the excitatory vasomotor mechanisms (adrenergic) are depressed during general anesthesia. The sympathetic vasodilator mechanisms are not affected except during enibomal and deep cyclopropane anesthesia. Sedation with morphine did not alter the circulatory responses to central and reflex stimulation.

Horwitz (49) studied the effects of intravenous ketamine hydrochloride and thiopental sodium anesthesia on left ventricular function in dogs. Average heart rate rose to 185 beats/min with ketamine and 147 beats/min with thiopental from a control value of 120 beats/min. Cardiac output increased with ketamine but was unchanged by thiopental; dp/dt of the left ventricle fell by 14% with thiopental but did not change with ketamine. The actions of ketamine may be explained on the basis of its ability to inhibit uptake of norepinephrine (50).

Korner and Whites (51) studied circulatory effects of chloralose plus urethane combinations, and that of pentobarbital in rabbits. The investigators could not find any significant differences in the local and autonomic effects of these two agents once the circulation became stable. In 1977, McGrath and Mackenzie (52) investigated the effect of thiopentone, althesin, pentobarbitone and ketamine on the cardiovascular system of the rabbit. Small doses of althesin and ketamine slightly potentiated responses of norepinephrine, while higher doses did not. They also produced small potentiation of the response to sympathetic

nerve stimulation at low doses and depressed these responses at higher doses. Pentobarbital (5 mg/kg) enhanced the effects of norepinephrine and attenuated the effects of sympathetic nerve stimulation in a similar manner to the higher doses of ketamine and althesin. Thiopentone, on the other hand, enhanced the effects of both norepinephrine (significantly at 4 mg/kg) and sympathetic nerve stimulation (significantly at 2 mg/kg). These workers concluded that in the absence of sympathetic nerve stimulation, each of the i.v. agents tested produced an immediate but short lived depression of the cardiovascular system. In pithed rabbits, a transient, dose-related cardiovascular depression was produced by each agent irrespective of whether vasomotor tone was present, whereas in decerebrate rabbits the corresponding cardiovascular depression was longer lasting. They also concluded that the cardiovascular depression produced by intravenous anesthetics in intact rabbits is due to a combination of central and peripheral effects.

In 1971, Sawyer et al., (53) studied the cardiovascular effects of halothane, methoxyflurane, pentobarbital and thiamylal in Hormel male swine. With each of these agents, cardiovascular depression was dose-related. The investigators also concluded that while the primary effect of halothane and methoxyflurane was probably due to myocardial depression, pentobarbital and thiamylal induced depression of myocardial function may be due to increased peripheral vascular resistance with subsequent decreased venous return to the heart.

Brezenoff (54) evaluated the effects of various anesthetic agents on the cardiovascular effects of norepinephrine in rats. In these

studies pressor response to norepinephrine was inhibited by diethyl ether, urethane and deep pentobarbitone anesthesia. It was unchanged by ketamine and potentiated by chloralose and light pentobarbitone anesthesia. Further, reflex bradycardia was potentiated by chloralose, blocked by ketamine and reduced by pentobarbitone, whereas it was either reduced or unchanged by ether or urethane.

Brown and Hilton (55,56) followed the effects of ether, alpha-chloralose, barbital sodium and pentobarbital on blood pressure and baroreceptor reflexes. Pressor response to bilateral carotid occlusion was completely inhibited by ether and it was attenuated by barbital sodium and pentobarbital. Dogs under chloralose showed a sharp rise in blood pressure and exhibited exaggerated baroreceptor reflexes. These investigators also reported that control blood pressure under chloralose is higher than under pentobarbital, ether or barbital sodium.

Wang and Borison (57) analyzed the carotid sinus reflex mechanisms in pentobarbital anesthetized dogs. These authors concluded that pentobarbital is a satisfactory anesthetic in the study of the carotid sinus cardiovascular reflex in spite of Heyman's (58) contention that chloralose has become the preferred anesthetic for these studies.

Verstraete (59) found morphine-thiopental less depressant on the carotid sinus reflexes in dogs than other anesthetics, while Prochnik et al., (60) showed that dogs under urethane produced greater pressor response to bilateral carotid occlusion than did dogs under ether or pentobarbital.

In 1963, Thomas (61) investigated the effect of sinus nerve stimulation on the chloralose anesthetized cat on blood pressure, heart

rate, muscle blood flow and vascular resistance. Blood pressure, heart rate and muscle vascular resistance increased during sinus nerve stimulation proportional to the frequency. Muscle blood flow remained essentially constant despite the pressure rise. Neil et al., (62) have shown that in the cat anesthetized with chloralose, stimulation of the sinus nerve with impulses of short duration has a pressor effect, suggesting that chemoreceptor function is predominant and that chloralose depressed baroreceptor function both centrally and peripherally, but concluded that the central action was the major factor in producing the pressor response to sinus nerve stimulation.

Vatner et al., (63) evaluated the effect of pentobarbital on carotid sinus nerve stimulation in dogs. The level of anesthesia did not significantly depress the decrease in heart rate and blood pressure observed during carotid sinus nerve stimulation. However, the time required for recovery of heart rate and arterial pressure became markedly prolonged by pentobarbital induction. These results led to the conclusion that the prolonged hypotension observed during carotid sinus nerve stimulation in anesthetized animals could be due to a supramedullary effect blocking the normal integration of information from other sensors in the cardiovascular system. This could be due to blockade of the aortic receptors that should respond to buffer the bradycardia and hypotension induced by carotid sinus nerve stimulation. Carotid sinus nerve stimulation in conscious dogs causes reduction in the resistance to blood flow in the coronary, iliac, mesenteric and renal vascular beds. Induction of pentobarbital anesthesia did not significantly

affect the decrease in resistance to blood flow in the renal and mesenteric beds. However, there was less vasodilitation in the iliac and coronary beds.

It is evident from the preceding background that all the anesthetics possess profound actions on various physiological systems, and it is expected that they may also influence pharmacology and mechanisms of drugs studied under these conditions.

More recently, Giles (64) studied the effect of prostaglandin E_1 on the systemic and pulmonary circulatory of intact mongrel dogs under the influence of pentobarbital and urethane anesthesia. Systemic infusion of prostaglandin E_1 in dogs anesthetized with sodium pentobarbital decreased systemic blood pressure. The cardiac index and heart rate were significantly increased with intra-arterial infusion but remained unchanged when prostaglandin was administered intravenously. Both intra-arterial (aortal) and intravenous infusion of prostaglandin E_1 produced a transient rise in mean pulmonary artery pressure; pulmonary venous pressure and pulmonary blood flow were essentially unchanged. Systemic infusion of PGE_1 in urethane anesthetized dogs caused a decrease in arterial pressure. Both intra-arterial (aortal) and intravenous infusion of PGE_1 produced a decrease in cardiac index and stroke volume. Heart rate increased when PGE_1 was given intra-arterially but decreased when it was given intravenously.

Jandhyala and Buckley (65) investigated the influence of various anesthetic agents on the effects of acute Δ^9-tetrahydrocannabinol (THC) on the blood pressure and heart rate of mongrel dogs. In dogs anesthetized with sodium pentobarbital, urethan or chloralose, Δ^9-THC reduced

heart rate and blood pressure significantly. However, in the animals anesthetized with a combination of morphine plus chloralose, Δ^9-THC produced a significant increase in heart rate, but a decrease in blood pressure. Further, THC reduced heart rate in conscious dogs and dogs sedated with morphine.

The purpose of this project was to investigate the influence of pentobarbital and morphine-chloralose anesthesia on the autonomic cardiovascular function and drug responses in mongrel dogs. Since these two anesthetics are more predominantly used in experimental animals for investigating the pharmacology and mechanisms of actions of experimental compounds, further information on these agents will be useful in evaluating their influence on drug action.

II. Materials and Methods

2.1 Studies on the hind limb vasculature

A total of 12 mongrel dogs weighing between 14-21 kg of either sex were utilized in this study. Six animals received pentobarbital, 35 mg/kg, i.v., and an additional six received morphine, 3 mg/kg, i.m. plus chloralose, 100 mg/kg, i.v. (in polyethylene glycol), as anesthetics. The trachea was intubated and the animals were placed on artificial respiration using a Bird respirator (Mark 7). They were prepared for the recording of blood pressure from the abdominal aorta via a catheter placed in the left femoral artery. The left femoral vein was catheterized for drug administration. In the animals anesthetized with pentobarbital heart rate was monitored by utilizing a cardiotachograph (Grass-7P 44) triggered by arterial pressure pulse and continuously recorded on a Grass polygraph. In the case of the animals anesthetized with morphine-chloralose, heart rate was calculated by counting the actual beats from the pressure pulse. The dogs were heparinized by administering 500 units/kg of heparin sodium. A midline incision was made into the abdominal cavity and the intestines retracted with gauze pads moistened with saline. The internal iliac artery was isolated and ligated to minimize collateral flow. The right femoral artery in the hind limb was isolated, the distal end was cannulated and perfused with blood from the aorta, which was taken by cannulating the proximal end of the femoral artery, using tygon tubing and a sigma motor pump, previously calibrated to yield the desired levels of flow. Since the flow was maintained at a constant rate, the perfusion pressure reflected peripheral vascular resistance and tone. Both aortic pressure and perfusion pressure were monitored by a Statham pressure transducer

(P23 Ac) and recorded on a Grass polygraph (Model 7). Both carotid arteries were isolated in the neck region. Bilateral carotid occlusion was performed by occluding these arteries and recording changes in blood pressure, perfusion pressure and heart rate over a period of 20 seconds. Also, responses to two doses of norepinephrine (0.5, 1.0 μg/kg, i.v.) were obtained in all the animals.

Pressure-flow relationships were obtained by altering flow rates and by recording perfusion pressure at each flow level. The flow rates in all preparations were 9.75, 24.7, 46.6, 73.3, 97.0 and 131.5 ml/min, and the order of selection of these flow rates was randomized in each experiment.

Denervation of the hind limb was accomplished by sectioning the sciatic and the femoral nerve trunks (66,67). Fifteen minutes after the limb denervation, alpha- and beta-adrenergic and muscurinic receptor activity in the hind limb was assessed by intra-arterial administration of norepinephrine and isoproterenol in doses of 0.1, 0.2, and 0.4 μg and acetylcholine in 0.5 and 1.0 μg doses into the inflow tube to the hind limb. Pressure flow curves in the denervated animals were recorded before and after the administration of phentolamine (3 mg/kg, i.v.).

2.2 Evaluation of sympathetic nerve function in the hind limb of mongrel dogs

A group of 10 mongrel dogs weighing between 13-19 kg of either sex were utilized in this study. Five dogs were anesthetized with 3 mg/kg morphine i.m., plus 100 mg/kg chloralose, i.v., given about 30 minutes after morphine, and five animals were anesthetized with sodium pentobarbital,

35 mg/kg, i.v. The trachea was intubated and the animals were placed on artificial respiration using a Bird respirator (Mark 7). The dogs were prepared for recording of blood pressure from the left femoral artery and the ipsilateral femoral vein was also catheterized. A Statham electromagnetic flow probe (2-3 mm diameter) was placed around the contralateral femoral artery and femoral blood flow was measured using a Statham electromagnetic flow meter (Sp-2202). A midline incision was made into the abdominal cavity and the intestines retracted with gauze pads moistened with normal saline.

The lumbar sympathetic chain (at L-5) was freed from the surrounding tissue and the peripheral fibers sectioned and placed over bipolar platinum electrodes. The nerve was stimulated using a Grass (SD-5) stimulator at supramaximal voltages (3-6 volts) with a pulse duration of 0.5 msec, using frequencies ranging from 0.25 to 8.0 pulses/sec for 15 sec. While there was a marked sympathetic stimulation at this level, there were no changes in aortic blood pressure, indicating that the decrease in femoral blood flow was mainly due to an increase in vascular resistance in the hind limb. Both blood flow and blood pressure were recorded on a Grass polygraph. Frequency-response curves to nerve stimulation at supramaximal voltage were obtained in both groups of animals.

2.3 The effect of morphine-chloralose and pentobarbital anesthesia on the reflexogenic bradycardia produced by norepinephrine and phenylephrine before and after the administration of clonidine

Eleven mongrel dogs weighing between 14-20 kg of either sex were used in these experiments. Five dogs were anesthetized with pentobarbital, 35 mg/kg, i.v., and 6 dogs were anesthetized with morphine,

3 mg/kg, i.m., followed 30 minutes later by 100 mg/kg of chloralose, i.v. in polyethylene glycol. After tracheal intubation, the dogs were placed on artificial respiration using a Bird respirator (Mark 7). Blood pressure and heart rate in both groups of anesthetized animals were recorded as described previously in section 2.1. Pressor and reflex bradycardic responses to norepinephrine (0.125, 0.25 and 0.5 μg/kg) and phenylephrine (1.25, 2.5 and 5.0 μg/kg) were obtained in both groups of animals prior to and following the administration of clonidine (20 μg/kg).

2.4 The effect of prostaglandin E_1 on blood pressure and heart rate in pentobarbital and morphine-chloralose anesthetized dogs

A groups of 10 mongrel dogs were used in this study. Five dogs were anesthetized with pentobarbital, 35 mg/kg, i.v., and 5 dogs were given 3 mg/kg morphine, i.m., plus 100 mg/kg chloralose, i.v. (in polyethylene glycol) as the anesthetic.

The trachea was intubated and the animals were placed on artificial respiration using a Bird respirator (Mark 7). The blood pressure was measured from the abdominal aorta through catheterization of the left femoral artery and was recorded on a Grass polygraph utilizing a pressure transducer (P23 Ac). The ipsilateral vein and the left common carotid artery were catheterized for the administration of experimental drugs. In the pentobarbital anesthetized animals, heart rate was monitored by utilizing a cardiotachograph as in section 2.1. In the case of the morphine-chloralose anesthetized animals, heart rate was calculated utilizing pressure-pulse as in section 2.1.

After a suitable stabilizing period, 3 doses of protaglandin E_1, 10, 20 and 40 ng/kg, i.v. and i.a., were administered to all animals and changes in blood pressure and heart rate observed.

2.5 The effect of different anesthetic agents on carotid sinus nerve stimulation

Eleven mongrel dogs weighing between 14-20 kg were utilized in this study. Five were anesthetized with pentobarbital, 35 mg/kg, i.v., and 6 animals were anesthetized with morphine, 3 mg/kg, i.m., plus 100 mg/kg chloralose (in polyethylene glycol) i.v. The trachea was intubated and the animals were placed on artificial respiration utilizing a Harvard respirator, then prepared for the recording of blood pressure from the left femoral artery. The ipsilateral femoral vein was cannulated for administration of drugs. Blood pressure and heart rate were recorded on a Grass polygraph as in section 2.1.

The carotid sinus nerve was isolated from between the internal and external carotid arteries and placed over a bipolar platinum electrode and was stimulated using a Grass (SD-5) stimulator at supramaximal voltage (3-6 volts) with a pulse duration of 0.5 msec, using frequencies ranging from 2-32 pulses/sec until a maximum response was obtained.

2.6 Effect of Δ^9-THC on systemic and pulmonary hemodynamics in morphine-chloralose anesthetized dogs

Five mongrel dogs weighing from 12-19 kg of either sex were anesthetized with 3 mg/kg morphine, i.m., plus 100 mg/kg chloralose dissolved in polyethylene glycol. The trachea was intubated and the animals were placed on artificial respiration using a Bird respirator

(Mark 7). The animals were prepared for the recording of blood pressure from a catheterized femoral artery using a Statham pressure transducer (P23 Dc); the heart rate was calculated from pressure pulse. Thoractomy was performed at the fourth intercostal space. The pericardium was incised to expose the heart, the aorta freed from surrounding tissue and a 11-14 mm Statham electromagnetic flow probe placed around the aorta to monitor cardiac output by means of a Statham electromagnetic flow meter (Sp-2202). A polyvinyl catheter was introduced into the left atrium via the apex to monitor left atrial pressure, and another was introduced into the pulmonary artery through a small branch to measure pulmonary arterial pressure, utilizing a venous pressure transducer (P23V). All parameters were recorded on a Grass polygraph (Model 7). Stroke volume (S.V.), right ventricular stroke work (R.V.S.W.), pulmonary vascular resistance (P.V.R.), and total peripheral resistance were mathematically calculated according to the formulas below. After obtaining the resting values for various parameters, 2.5 mg/kg of Δ^9-tetrahydrocannabinol (Δ^9-THC) was injected directly into the brachial vein and the effect of Δ^9-THC on various cardiovascular functions was recorded at 5, 10, 15, 30, 60 and 90 minutes.

Stroke volume (ml/beat)

$$= \frac{\text{cardiac output (ml/min)}}{\text{heart rate (beats/min)}}$$

Right ventricular stroke work (R.V.S.W.)

$$= \frac{\text{S.V. x PAP x 13.6 gram-meter}}{1000}$$

Pulmonary vascular resistance (P.V.R.)

$$= \frac{\text{(PAP - LAP) x 1332 dynes sec/cm}^5}{\text{cardiac output (ml/sec)}}$$

Total peripheral resistance (T.P.R.)

$$= \frac{\text{mean blood pressure x 1332}}{\text{cardiac output (ml/sec)}}$$

PAP = pulmonary arterial pressure

LAP = left atrial pressure

CO = cardiac output

2.7 Effect of anesthetics and SQ-14,225 (D-3-mercapto-2-methyl-propanoyl-L-proline, an angiotensin converting enzyme inhibitor) on plasma renin activity

Mongrel dogs of either sex weighing between 16-26 kg were used in this study. Five were anesthetized with pentobarbital (35 mg/kg, i.v.) and an additional five with morphine (3 mg/kg, i.m.) plus chloralose (100 mg/kg, i.v.). The trachea was intubated and the animals were put on artificial respiration using a Bird respirator. The left femoral artery was cannulated for the measurement of blood pressure using a Statham pressure transducer and the left femoral vein was cannulated for the injection of drugs and withdrawal of blood samples. Heart rate was obtained as described in section 2.1. All parameters were recorded on a Grass polygraph. Approximately 5 ml of blood was taken from the brachial vein before the administration of the anesthetic. Three other blood samples were withdrawn from the femoral vein at 15, 30 and 60 minutes after the administration of the anesthesia. SQ-14,225 (3 mg/kg, i.v.) was administered and an additional three blood samples were withdrawn at 15, 30 and 60 minutes after the administration of the drug.

The blood was transferred into a pre-chilled tube containing approximately 5 mg EDTA Na. The tube was immediately placed in ice and centrifuged in a refrigerated centrifuge to recover the plasma. Plasma renin

activity was determined by radioimmunoassay utilizing an Angiotensin-I Immunotope Kit (E. R. Squibb & Sons, Inc.). The quantities of angiotensin I formed during the 3 hours incubation period in the presence of angiotensinase inhibitors was determined by radioimmunoassay (68). The results are expressed in terms of ng of angiotensin I generated per ml per one hour of incubation (ng/ml/hr).

Statistical Analysis: All the data are reported as mean ± standard error of the mean (S.E.M.). Paired t-test was utilized in all the calculations to estimate significant changes within the same group, whereas differences between the means of two groups were evaluated by using student's t-test. The difference between the means was considered statistically significant when $p < 0.05$.

Drugs: The drugs used in this study and source were:

1. Pentobarbital sodium: Sigma Chemical Company.
2. Alpha-chloralose (99%): Aero Chemical Corporation.
3. Morphine Sulfate: ESI Pharmaceuticals.
4. Levophed bitartrate (norepinephrine): Wimthrop Laboratories.
5. Isoproterenol hydrochloride: Winthrop Laboratories.
6. Acetylcholine chloride: Sigma Chemical Company.
7. Neo-Synephrine (phenylephrine hydrochloride): Winthrop Laboratories.
8. Clonidine hydrochloride: Boehringer Ingelheim, Ltd.
9. Prostaglandin E_1: The Upjohn Company.
10. Δ^9-tetrahydrocannabinol: supplied by the National Institute of Mental Health.

III. Results

3.1 Hind limb vasculature studies

Figures 1-6 represent results obtained from a study done on the hind limb under pentobarbital and morphine-chloralose anesthesia.

Figures 1 and 2 show the perfusion pressure at different levels of blood flow in the innervated and denervated leg and after the administration of an alpha-blocker (phentolamine) in pentobarbital and morphine-chloralose anesthetized animals, respectively. Perfusion pressure curves were higher at all levels of flow in the morphine-chloralose anesthetized animals than in pentobarbital anesthetized dogs and perfusion pressure was significantly higher at 50-132 ml/min blood flow rates in these morphine-chloralose anesthetized dogs. However, the magnitude of decrease in the perfusion pressure at various flow rates observed after denervation was almost identical in both groups of anesthetized dogs. Further, the decrease in perfusion pressure after denervation, plus the administration of an alpha-blocker, was of the same magnitude in both groups of animals. Denervation of the hind limb and administration of an alpha-blocker resulted in a significant shift of the pressure flow curve (Figure 3) to the right in the pentobarbital-anesthetized dogs in comparison to that of the morphine-chloralose anesthetized animals. The lower perfusion pressure in the pentobarbital anesthetized dog's hind limb after denervation plus the administration of an alpha-blocker clearly suggests a lower resistance to blood flow in these animals than in the ones anesthetized with morphine-chloralose.

Figure 4 shows the increase in both blood pressure and hind leg perfusion pressure produced in response to bilateral carotid occlusion

Figure 1. Pressure flow curves in the hind limb of pentobarbital anesthetized dogs before (o———o), after (●———●) denervation and after denervation plus α-blocker (Δ———Δ); N = 6.

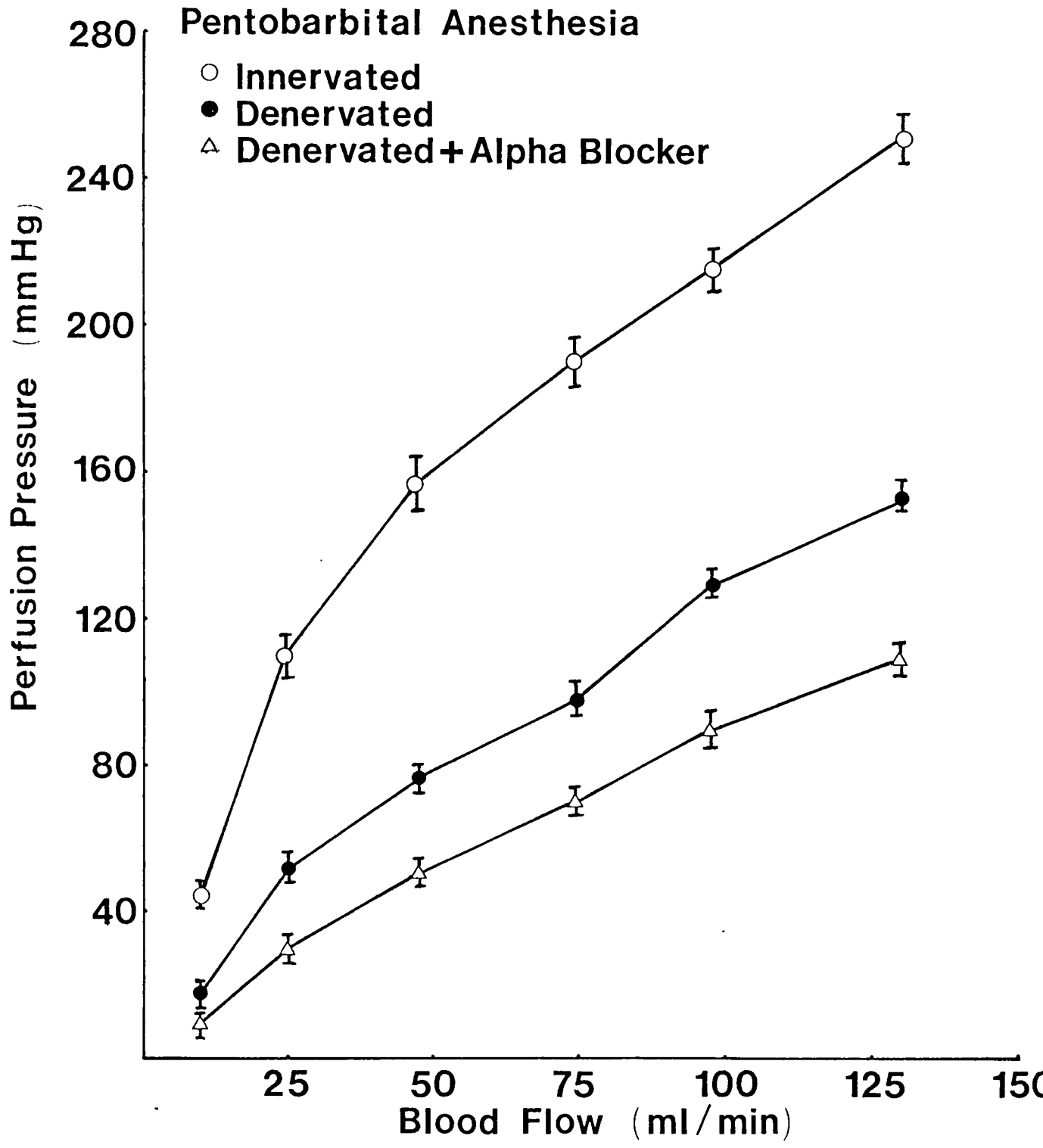

Pentobarbital Anesthesia
Innervated
Denervated
Denervated+Alpha Blocker
Perfusion Pressure (mm Hg)
280
240
200
160
120
80
40
25
50
75
100
125
150
Blood Flow (ml/min)

Figure 2. Pressure flow curves in the hind limb of morphine-chloralose anesthetized dogs before (□——□), after (■——■) denervation and after denervation plus α-blocker (Δ——Δ); N = 6.

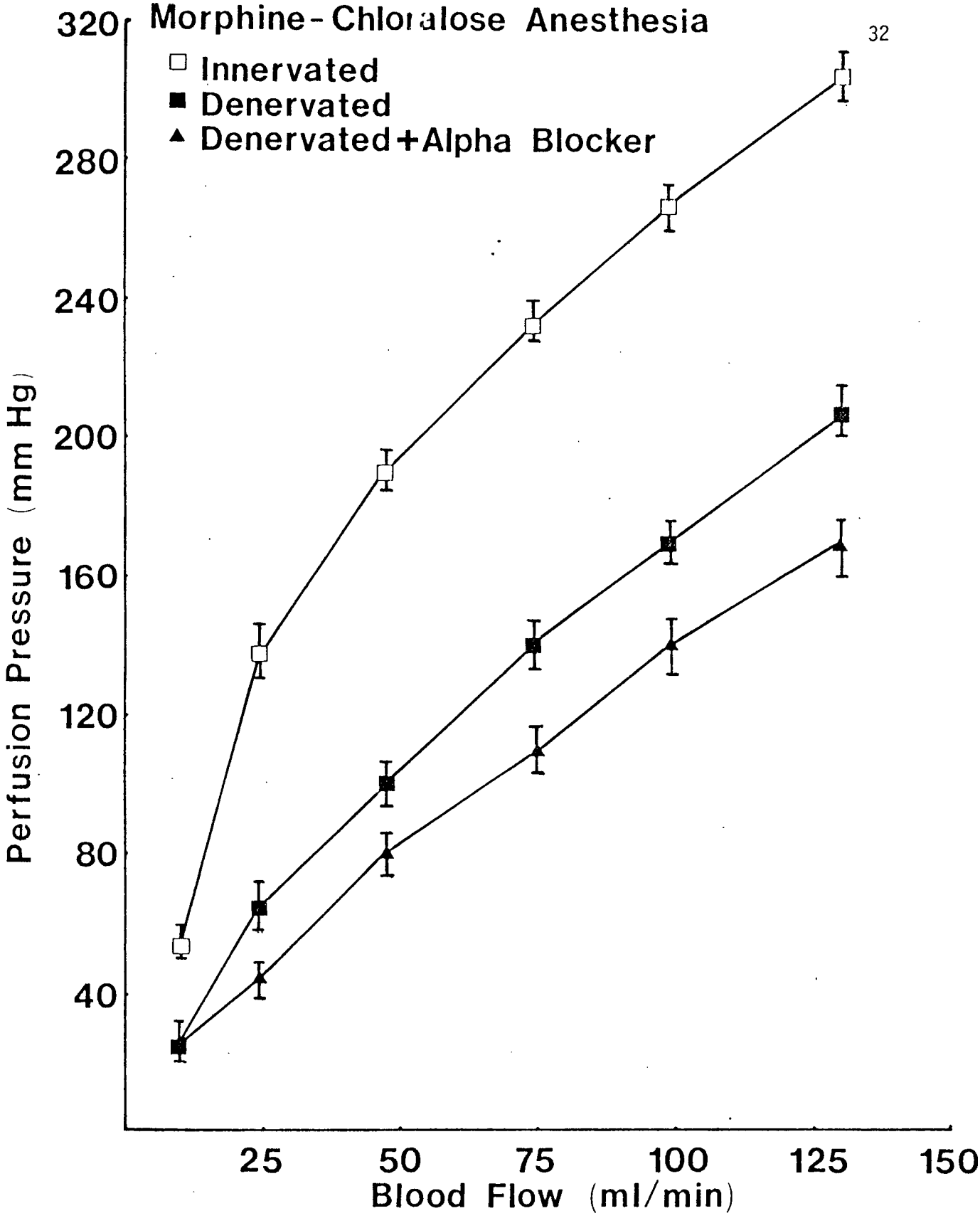

Morphine-Chloralose Anesthesia
Innervated
Denervated
Denervated+Alpha Blocker
320
280
240
200
160
120
80
40
Perfusion Pressure (mm Hg)
25
50
75
100
125
150
Blood Flow (ml/min)

Figure 3. Pressure flow curves in the hind limb of pentobarbital (o———o) and morphine-chloralose (□———□) anesthetized dogs after denervation plus α-blocker. (N = 6 each group)

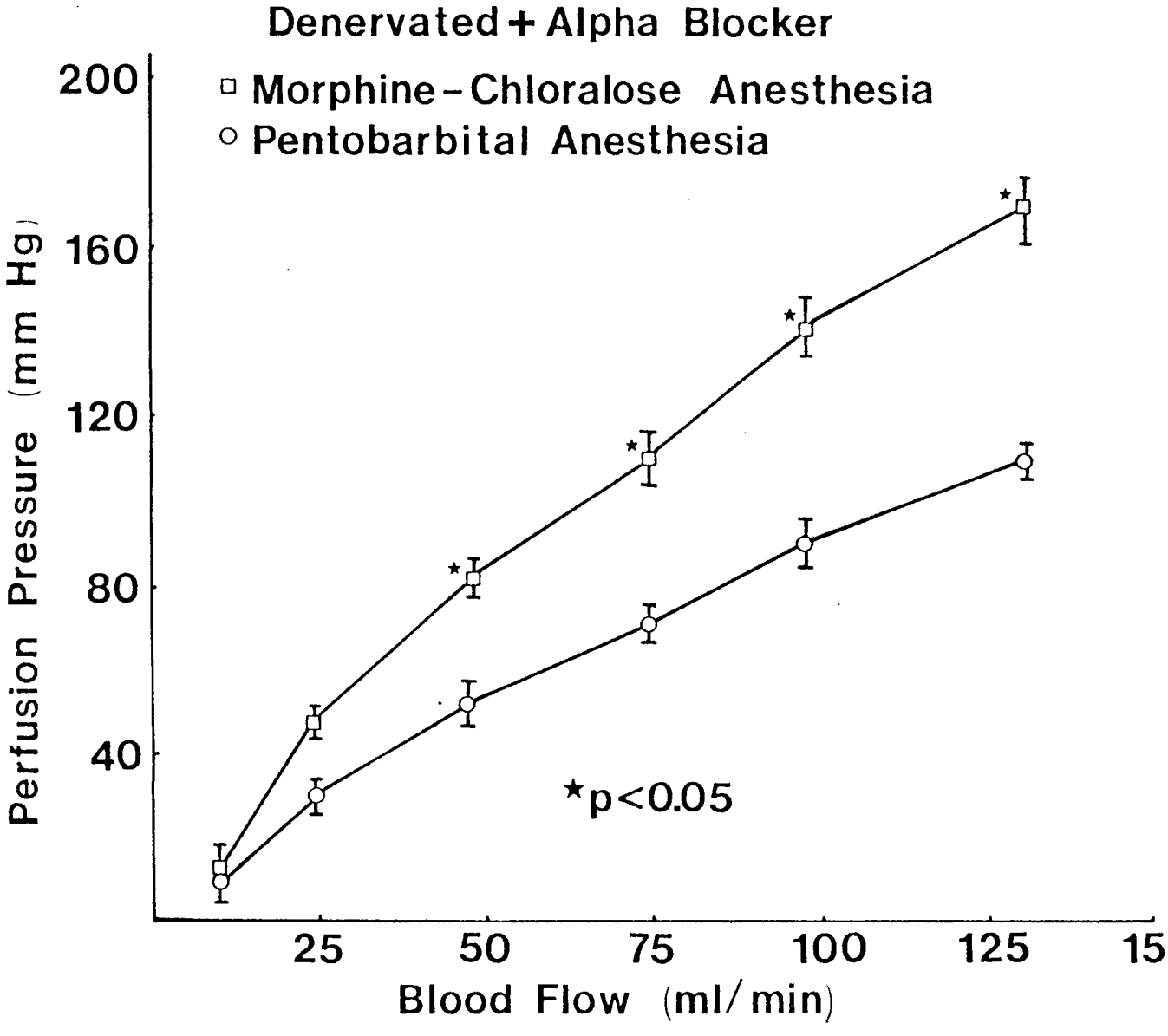
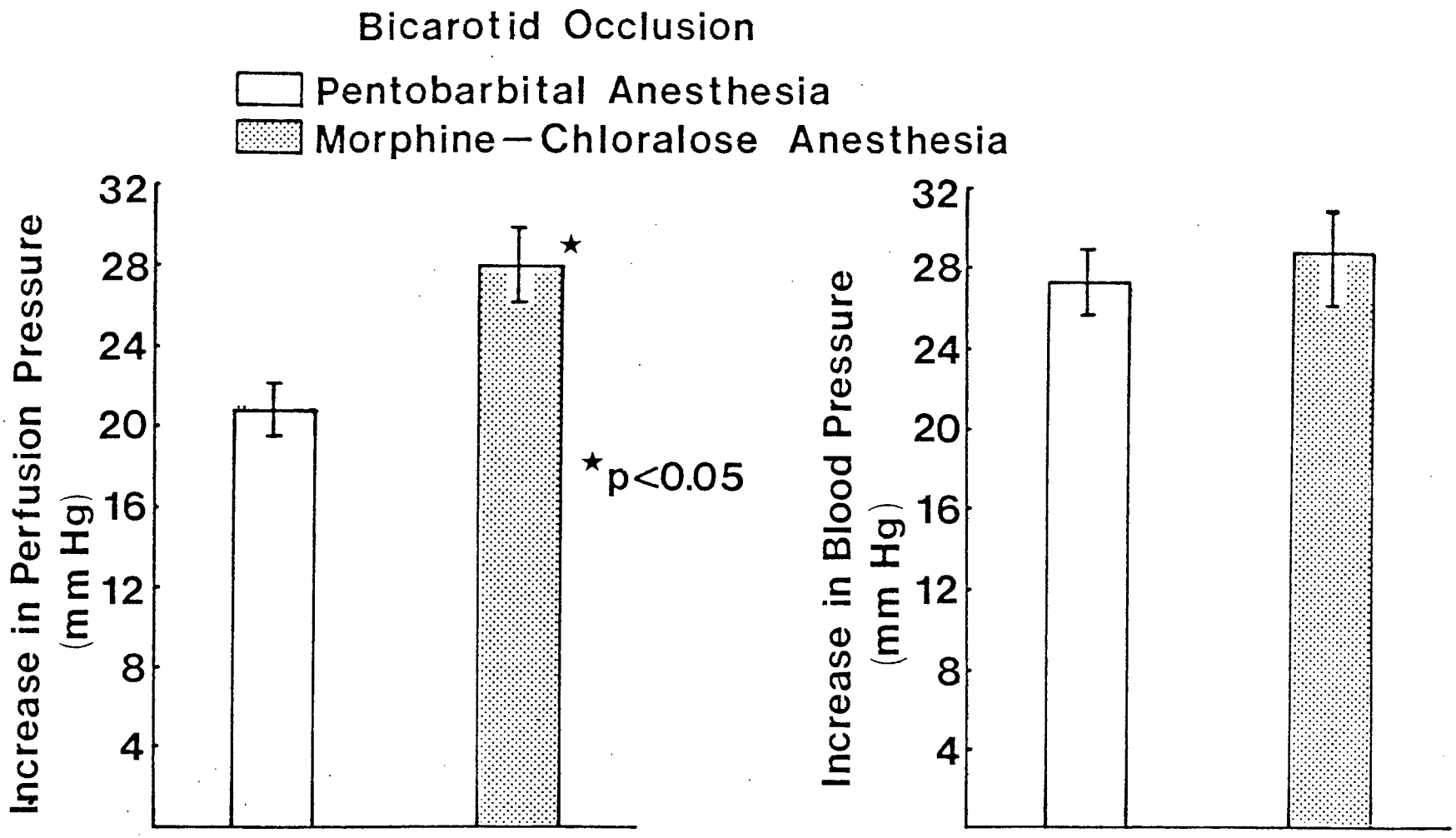

Denervated + Alpha Blocker
□ Morphine-Chloralose Anesthesia
○ Pentobarbital Anesthesia
★p<0.05
Perfusion Pressure (mm Hg)
200
160
120
80
40
25
50
75
100
125
150
Blood Flow (ml/min)

Figure 4. Increase in perfusion pressure and blood pressure to bilateral carotid occlusion (20 sec) in pentobarbital (□) and morphine-chloralse (▦) anesthetized dogs. (N = 6 each group)


Bicarotid Occlusion
Pentobarbital Anesthesia
Morphine—Chloralose Anesthesia
Increase in Perfusion Pressure (mm Hg)
32
28
24
20
16
12
8
4
★
★p<0.05
Increase in Blood Pressure (mm Hg)
32
28
24
20
16
12
8
4

in both groups of animals. There was a significantly greater increase in the hind leg perfusion pressure to bilateral carotid occlusion in morphine-chloralose anesthetized dogs compared to the dogs under pentobarbital anesthesia ($p < 0.05$).

Figure 5 represents the increase in blood pressure and reflex decrease in perfusion pressure in response to 0.5 and 1.0 μg/kg, i.v. norepinephrine. The increase in blood pressure in response to 0.5 μg/kg, i.v. norepinephrine is about the same in both groups of animals; however, the increase in blood pressure is significantly larger ($p < 0.05$) in response to 1.0 μg/kg, i.v. norepinephrine in the pentobarbital anesthetized dogs. The reflex decrease in perfusion pressure was larger in dogs under morphine-chloralose anesthesia in response to both 0.5 and 1.0 μg/kg, i.v. norepinephrine. However, the decrease in perfusion pressure in morphine-chloralose anesthetized dogs was only significant at 1.0 μg/kg norepinephrine ($p < 0.05$).

Figure 6 shows changes in perfusion pressure in response to several doses of norepinephrine and isoproterenol given intra-arterially. In response to norepinephrine, hind leg perfusion pressure showed a significantly greater increase in the dogs anesthetized with morphine-chloralose than in dogs anesthetized with pentobarbital. The decrease in perfusion pressure observed after i.a. isoproterenol was similar in both groups of anesthetized dogs.

3.2 Sympathetic nerve function in the hind limb

Sympathetic nerve function in the hind limb was studied by stimulating the lumbar sympathetic chain at supramaximal voltage,

Figure 5. Increase in blood pressure and decrease in perfusion pressure in response to norepinephrine (0.5, 1.0 μg/kg, i.v.) in pentobarbital (o———o) and morphine-chloralose (□———□) anesthetized dogs. (N = 6 each group)

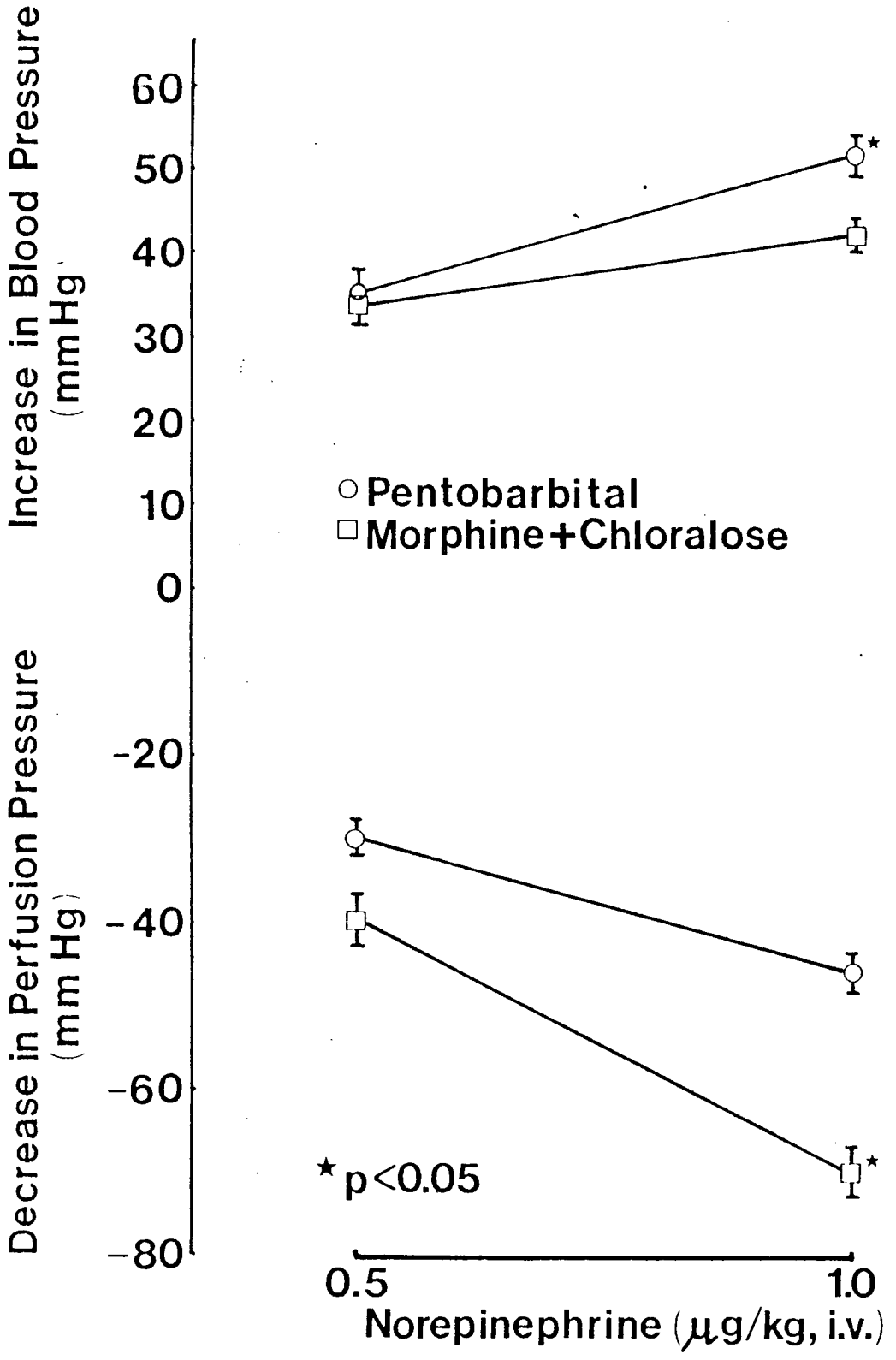
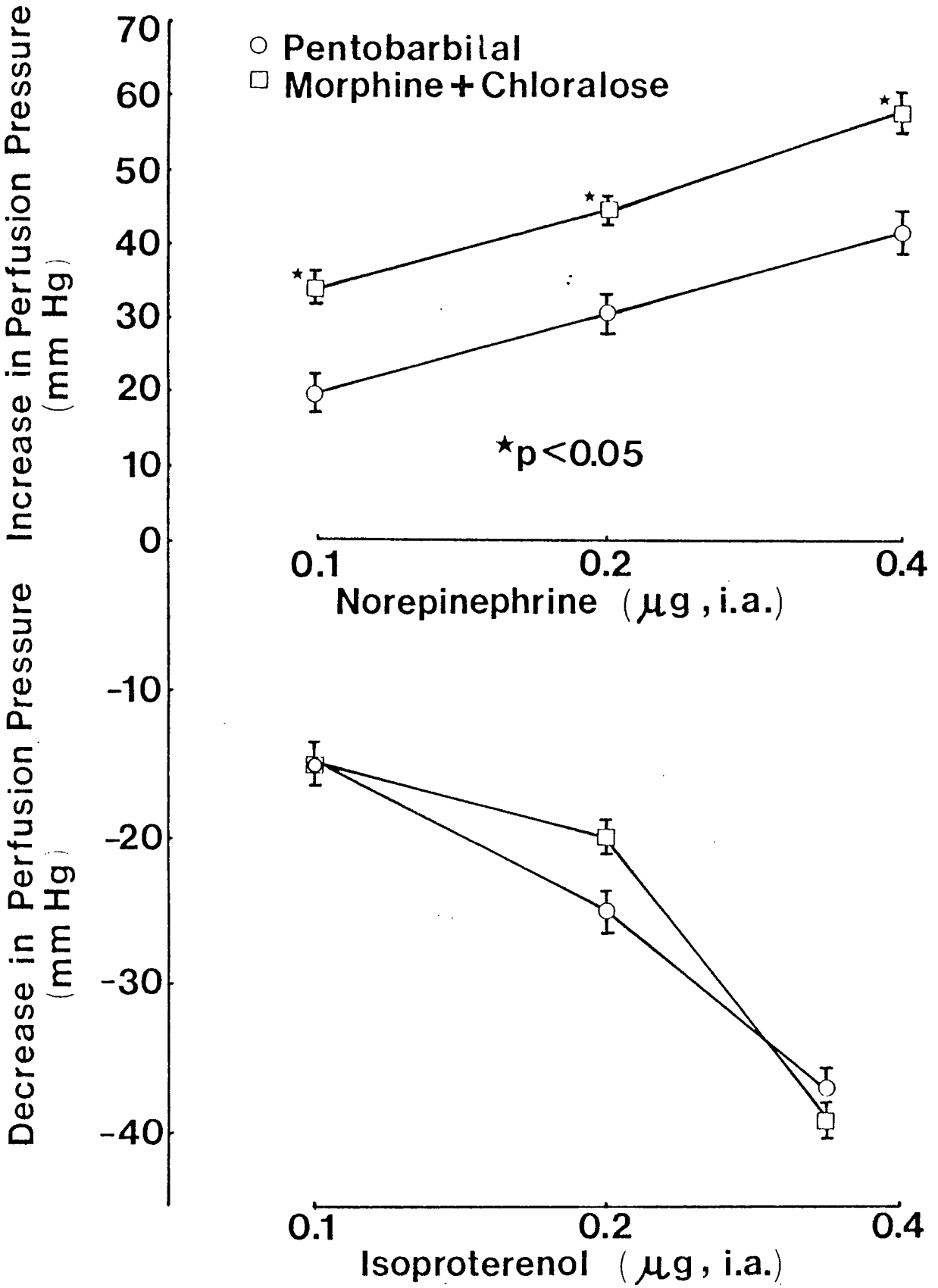

Increase in Blood Pressure (mmHg)
Decrease in Perfusion Pressure (mmHg)
60
50
40
30
20
10
0
-20
-40
-60
-80
○ Pentobarbital
□ Morphine+Chloralose
★ p<0.05
0.5
1.0
Norepinephrine (μg/kg, i.v.)

Figure 6. Increase in perfusion pressure in response to norepinephrine (.1, .2 and .4 µg, i.a.) and decrease in perfusion pressure in response to isoproterenol (.1, .2 and .4 µg, i.a.) in pentobarbital (o——o), and morphine-chloralose (□——□) anesthetized dogs. (N = 6 each group)


○ Pentobarbital
□ Morphine + Chloralose
Increase in Perfusion Pressure (mm Hg)
Decrease in Perfusion Pressure (mm Hg)
70
60
50
40
30
20
10
0
-10
-20
-30
-40
★p<0.05
0.1
0.2
0.4
Norepinephrine (μg, i.a.)
Isoproterenol (μg, i.a.)

0.5 msec duration, at various frequencies in dogs anesthetized with pentobarbital and morphine-chloralose.

Figure 7 shows the decrease in blood flow in the hind limb in response to the lumbar sympathetic chain stimulation. There is significantly greater ($p < 0.05$) decrease in blood flow to the hind limb in the dogs anesthetized with pentobarbital sodium than in the dogs anesthetized with morphine-chloralose.

3.3 Effect of morphine-chloralose and pentobarbital anesthesia on reflexogenic bradycardia

The effect of morphine-chloralose and pentobarbital anesthesia on the reflexogenic bradycardia was studied by administering three doses (0.125, 0.25 and 0.5 μg/kg, i.v.) of norepinephrine and three doses (1.25, 2.5 and 5.0 μg/kg, i.v.) of phenylephrine before and after clonidine (20 μg/kg, i.v.). The percent increase in blood pressure is almost equal in both groups at the lower dose (.125 μg/kg), but is significantly greater at the larger dose (0.5 μg/kg) in the dogs anesthetized with morphine-chloralose. However, the percent decrease in heart rate was significantly greater in the morphine-chloralose anesthetized dogs at all doses (Figure 8).

Figure 9 shows percent changes in blood pressure and heart rate in response to phenylephine in pentobarbital and morphine-chloralose anesthetized dogs. Percent increase in blood pressure was approximately the same in both groups of anesthetized dogs, but the percent decrease in heart rate was always significantly greater ($p < 0.05$) in dogs anesthetized with morphine-chloralose at all the dose levels of phenylephrine.

Figure 7. Decrease in blood flow to the hind limb in response to stimulation of the lumbar sympathetic chain in pentobarbital (o———o) and morphine-chloralose (□———□) anesthetized dogs.

Pentobarbital: Resting blood flow - 71.2 ± 3.2 ml/min.

Morphine-chloralose: Resting blood flow - 56.4 ± 8.5 ml/min.

(N = 5 each group)

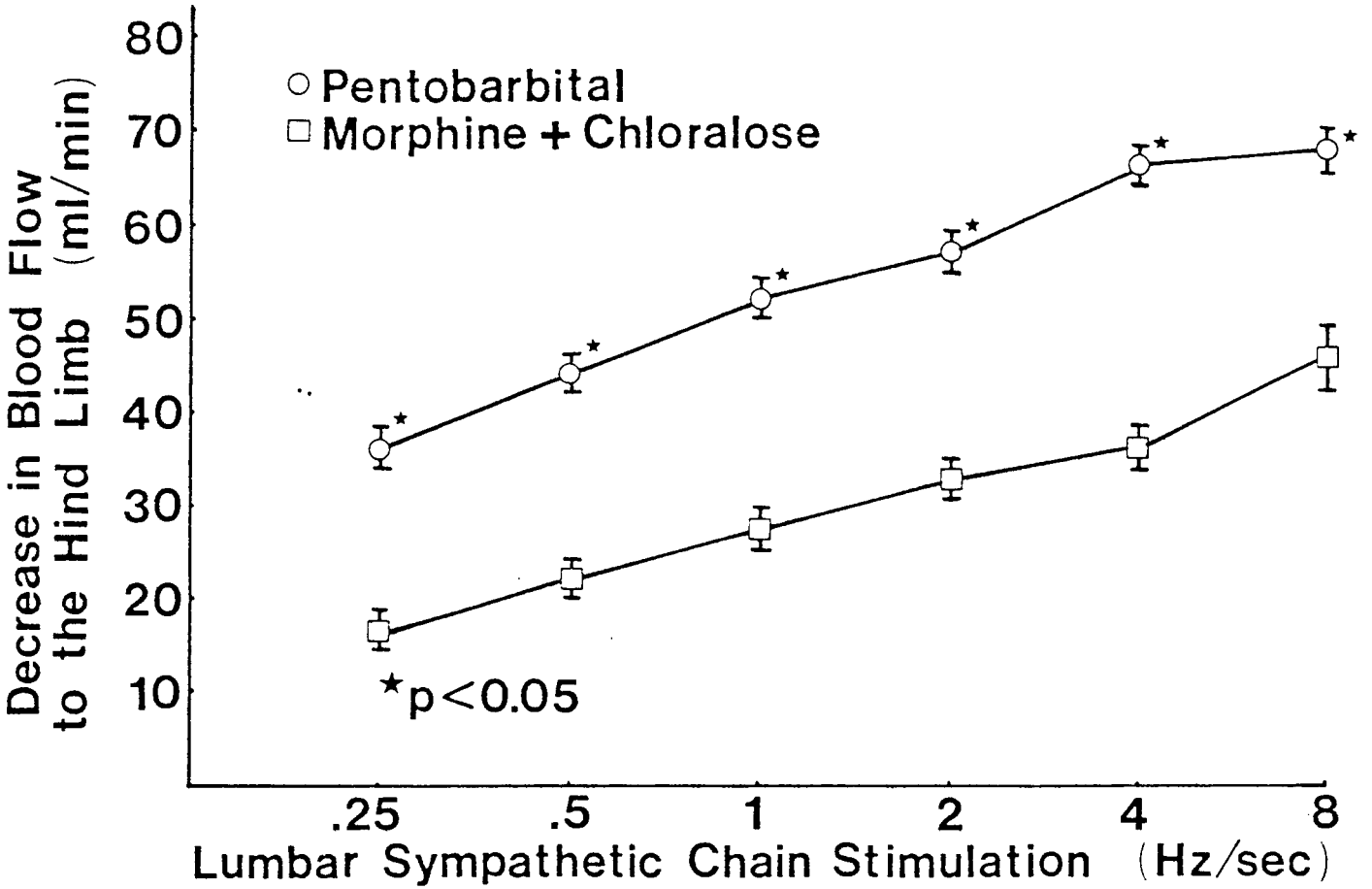

Decrease in Blood Flow to the Hind Limb (ml/min)
80
70
60
50
40
30
20
10
○ Pentobarbital
□ Morphine + Chloralose
★p<0.05
.25
.5
1
2
4
8
Lumbar Sympathetic Chain Stimulation (Hz/sec)

Figure 8. Percent increase in blood pressure and percent decrease in heart rate in response to norepinephrine (.125, .25 and .5 μg/kg, i.v.) in pentobarbital (o———o) and morphine-chloralose (□———□) anesthetized dogs.

Pentobarbital: Resting MBP = 141.2 ± 10.7 mm Hg

Resting HR = 154.8 ± 8.2 beats/min (N = 5)

Morphine-chloralose: Resting MBP = 98.6 ± 8.1 mm Hg

Resting HR = 81.3 ± 6.6 beats/min (N = 6)

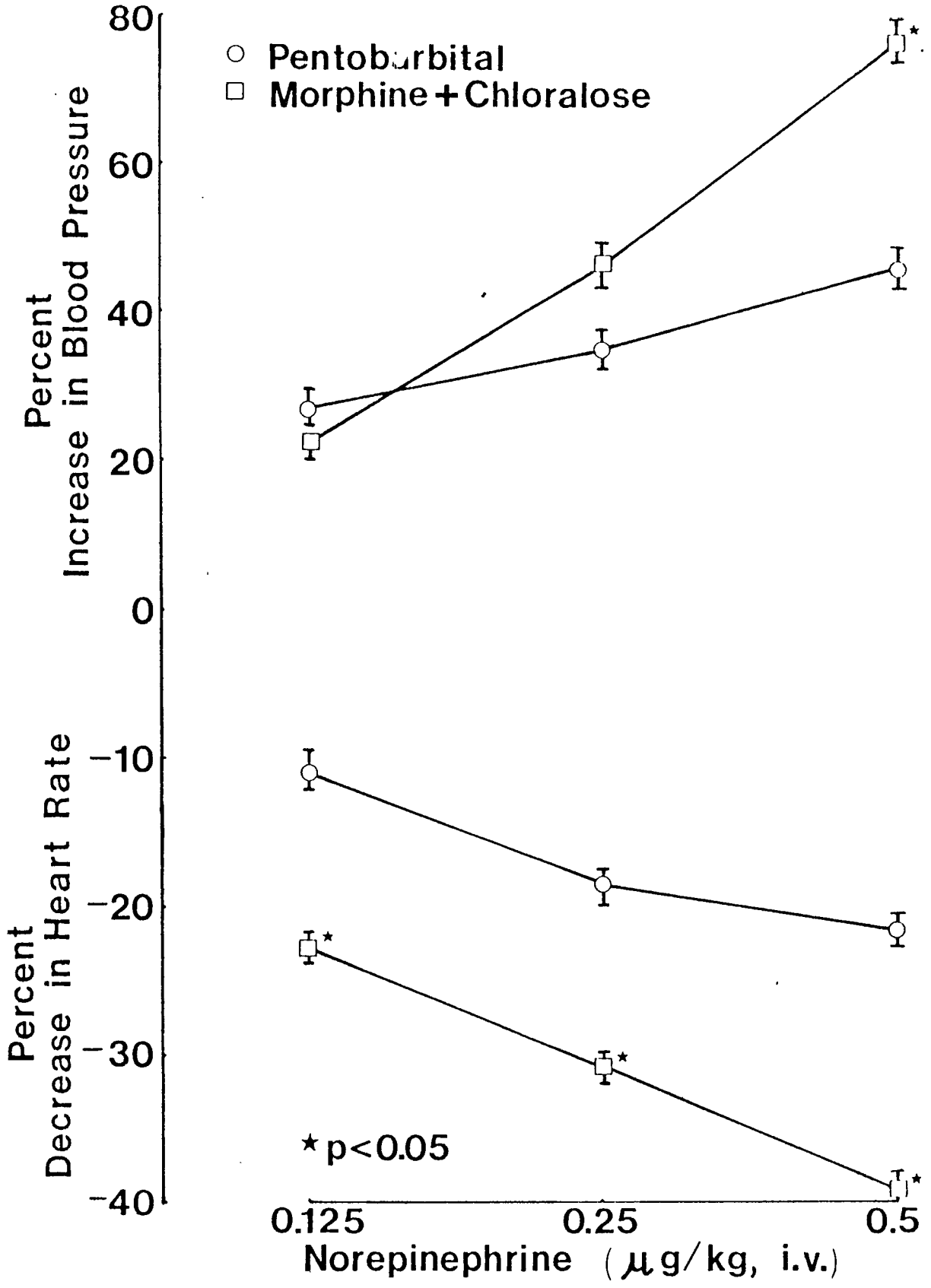

Pentobarbital
Morphine + Chloralose
Percent Increase in Blood Pressure
Percent Decrease in Heart Rate
80
60
40
20
0
−10
−20
−30
−40
★ p<0.05
0.125
0.25
0.5
Norepinephrine (μg/kg, i.v.)

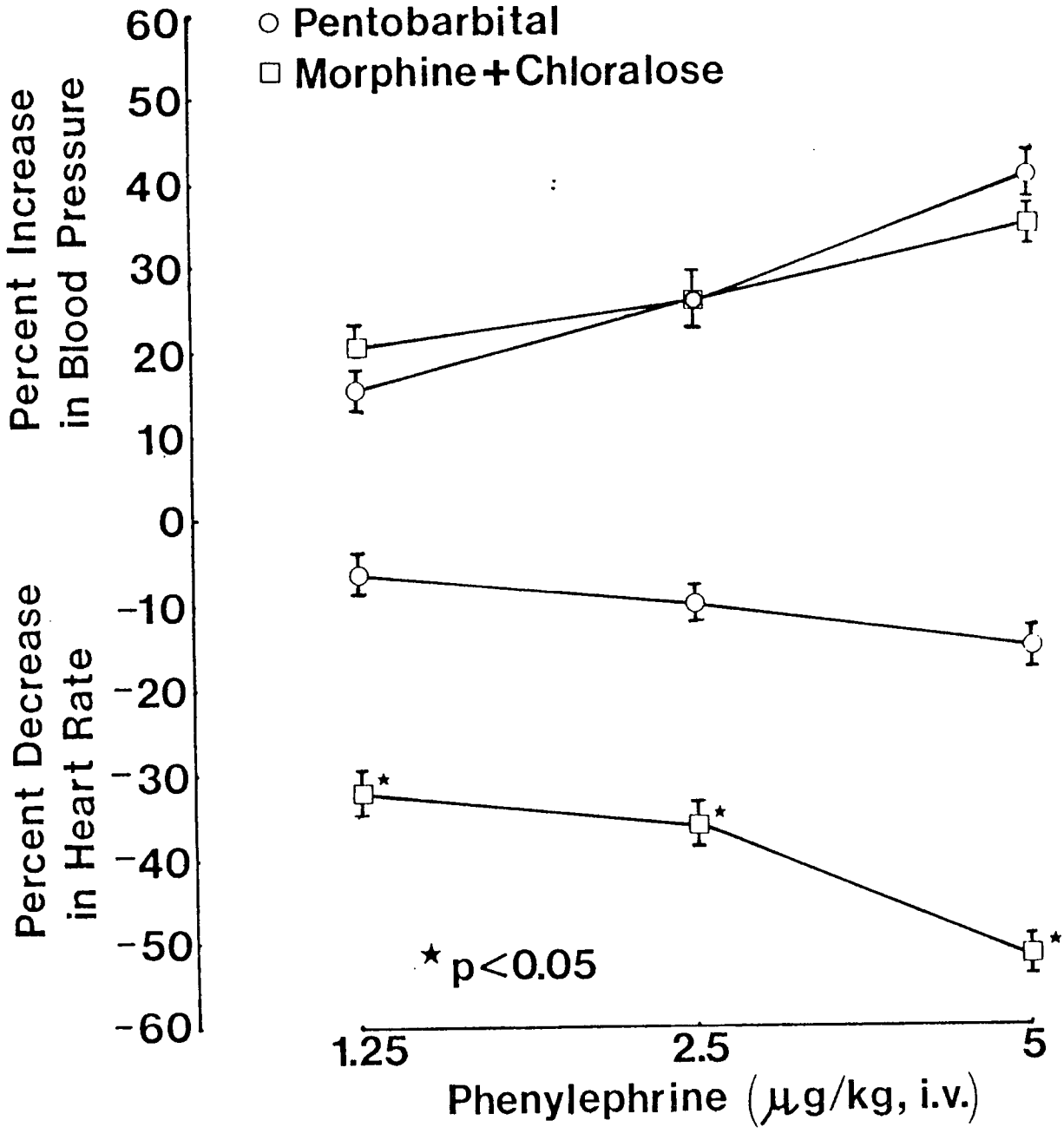
Figure 9. Percent increase in blood pressure and percent decrease in heart rate in response to phenylephrine (1.25, 2.5 and 5.0 µg/kg, i.v.) in pentobarbital (o———o) and morphine-chloralose (□———□) anesthetized dogs.

Pentobarbital: Resting MBP = 141.2 ± 10.7 mm Hg

Resting HR = 154.8 ± 8.2 beats/min (N = 5)

Morphine-chloralose: Resting MBP = 98.6 ± 8.1 mm Hg

Resting HR = 81.3 ± 6.6 beats/min (N = 6)


○ Pentobarbital
□ Morphine+Chloralose
Percent Increase in Blood Pressure
Percent Decrease in Heart Rate
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
★ p<0.05
1.25
2.5
5
Phenylephrine (μg/kg, i.v.)

Pressor responses to norepinephrine were significantly greater in the morphine-chloralose group than the pentobarbital group (Figure 10). Clonidine administration (20 μg/kg, i.v.) did not produce any alteration in norepinephrine induced pressor responses in either group. However, reflex bradycardia responses to norepinephrine were potentiated in the pentobarbital group, but not in the dogs anesthetized with morphine-chloralose (Figure 10). In contrast, reflex bradycardic responses to phenylephrine were potentiated by clonidine in both groups (Figure 11). Similarly, pressor reponses to phenylephrine were essentially identical in the two anesthetic groups prior to clonidine administration. Further, clonidine inhibited pressor responses to phenylephrine only in the pentobarbital group (Figure 11).

3.4 The effect of prostaglandin E_1 on blood pressure and heart rate

The effect of prostalgandin E_1 on blood pressure and heart rate was studied by administering PGE_1 i.v. and i.a. (10, 20 and 40 ng/kg).

Prostaglandin E_1 given i.v. in the above doses caused no change in either blood pressure or heart rate in dogs under the two different anesthetics.

Figure 12 shows the changes in blood pressure and heart rate elicited by administration of PGE_1, i.a. Protaglandin E_1, i.a. caused no change in blood pressure in dogs anesthetized with morphine-chloralose; however, there was a very marked decrease in the blood pressure of the animals anesthetized with pentobarbital. The magnitude of the decrease in blood pressure was approximately 25 mm Hg when 40 ng/kg of PGE_1 was administered. Dogs anesthetized with pentobarbital and morphine-chloralose showed

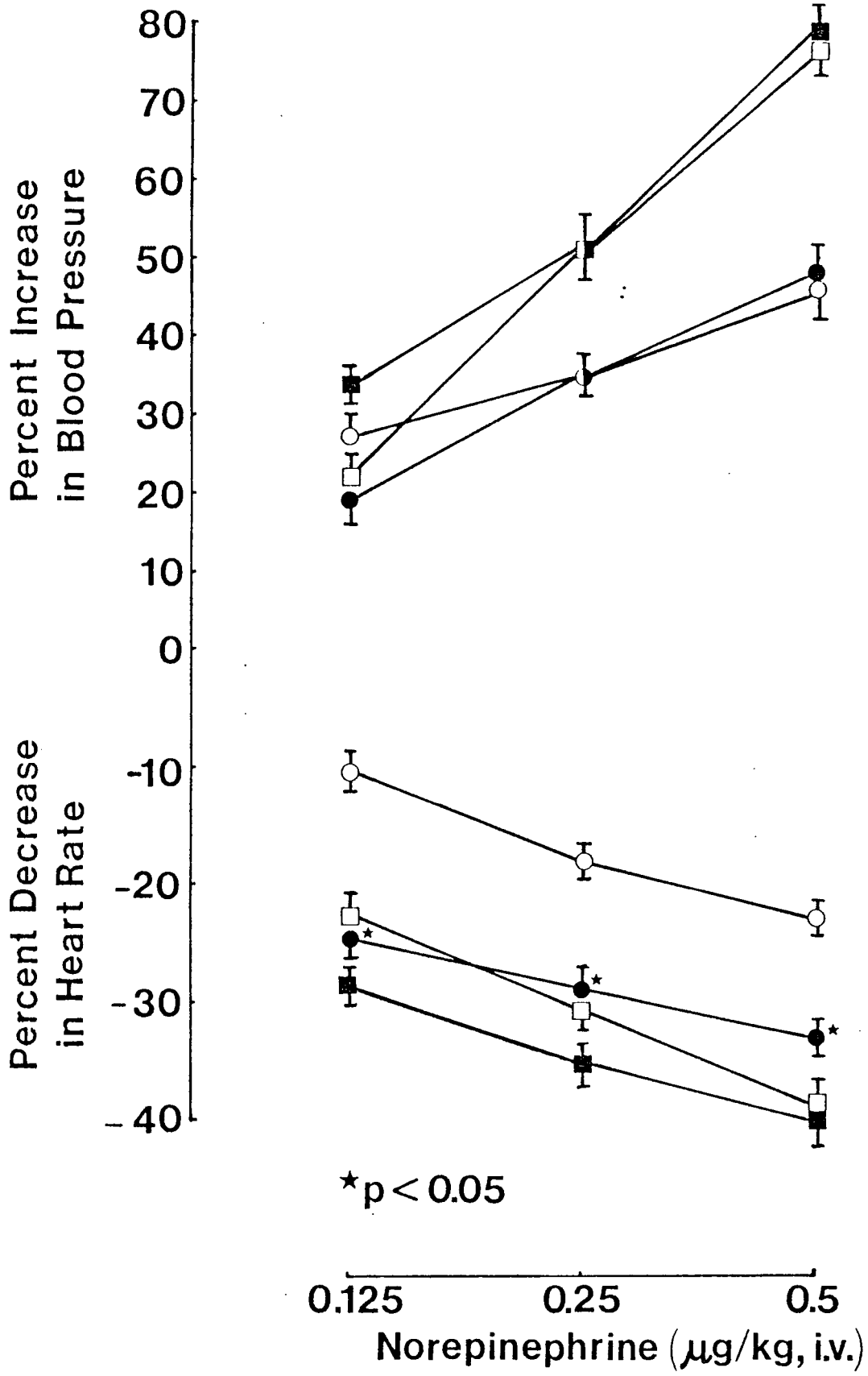
Figure 10. Percent increase in blood pressure and percent decrease in heart rate in response to norepinephrine (.125, .25 and .5 μg/kg, i.v.) in pentobarbital anesthetized dogs before (o———o) and after (●———●) clonidine, 20 μg/kg, i.v., and in morphine-chloralose anesthetized dogs before (□———□) and after (■———■) clonidine.

Pentobarbital: Resting MBP = 140 ± 7.4 mm Hg

Resting HR = 147 ± 6.8 beats/min (N = 5)

Morphine-chloralose: Resting MBP = 94 ± 6.1 mm Hg

Resting HR = 74 ± 7.8 beats/min (N = 6)


80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
Percent Increase in Blood Pressure
Percent Decrease in Heart Rate
★p < 0.05
0.125
0.25
0.5
Norepinephrine (μg/kg, i.v.)

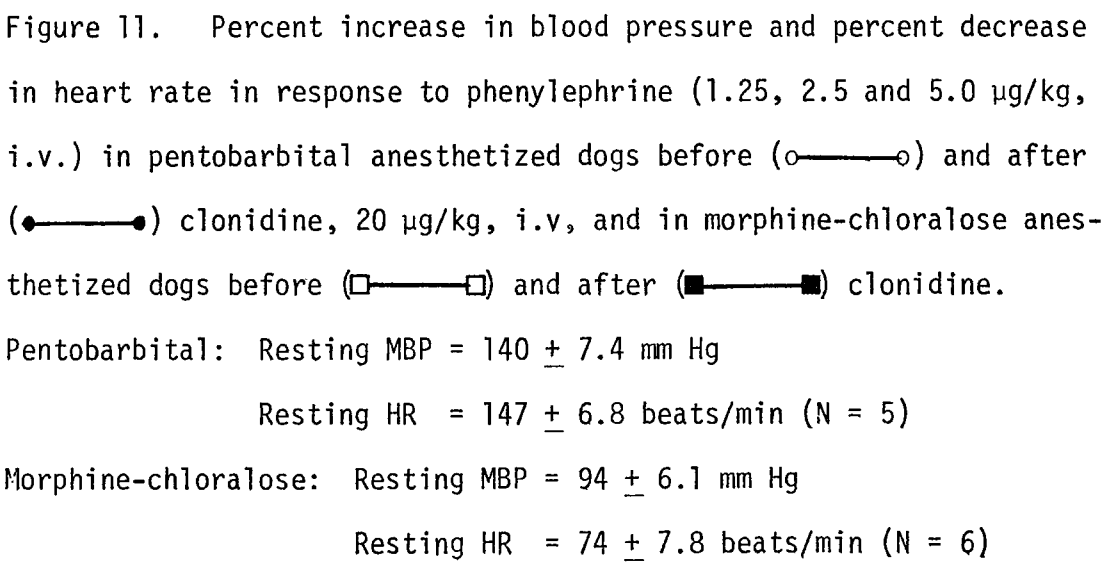


Figure 11. Percent increase in blood pressure and percent decrease in heart rate in response to phenylephrine (1.25, 2.5 and 5.0 µg/kg, i.v.) in pentobarbital anesthetized dogs before (o———o) and after (●———●) clonidine, 20 µg/kg, i.v, and in morphine-chloralose anesthetized dogs before (□———□) and after (■———■) clonidine.

Pentobarbital: Resting MBP = 140 ± 7.4 mm Hg

Resting HR = 147 ± 6.8 beats/min (N = 5)

Morphine-chloralose: Resting MBP = 94 ± 6.1 mm Hg

Resting HR = 74 ± 7.8 beats/min (N = 6)

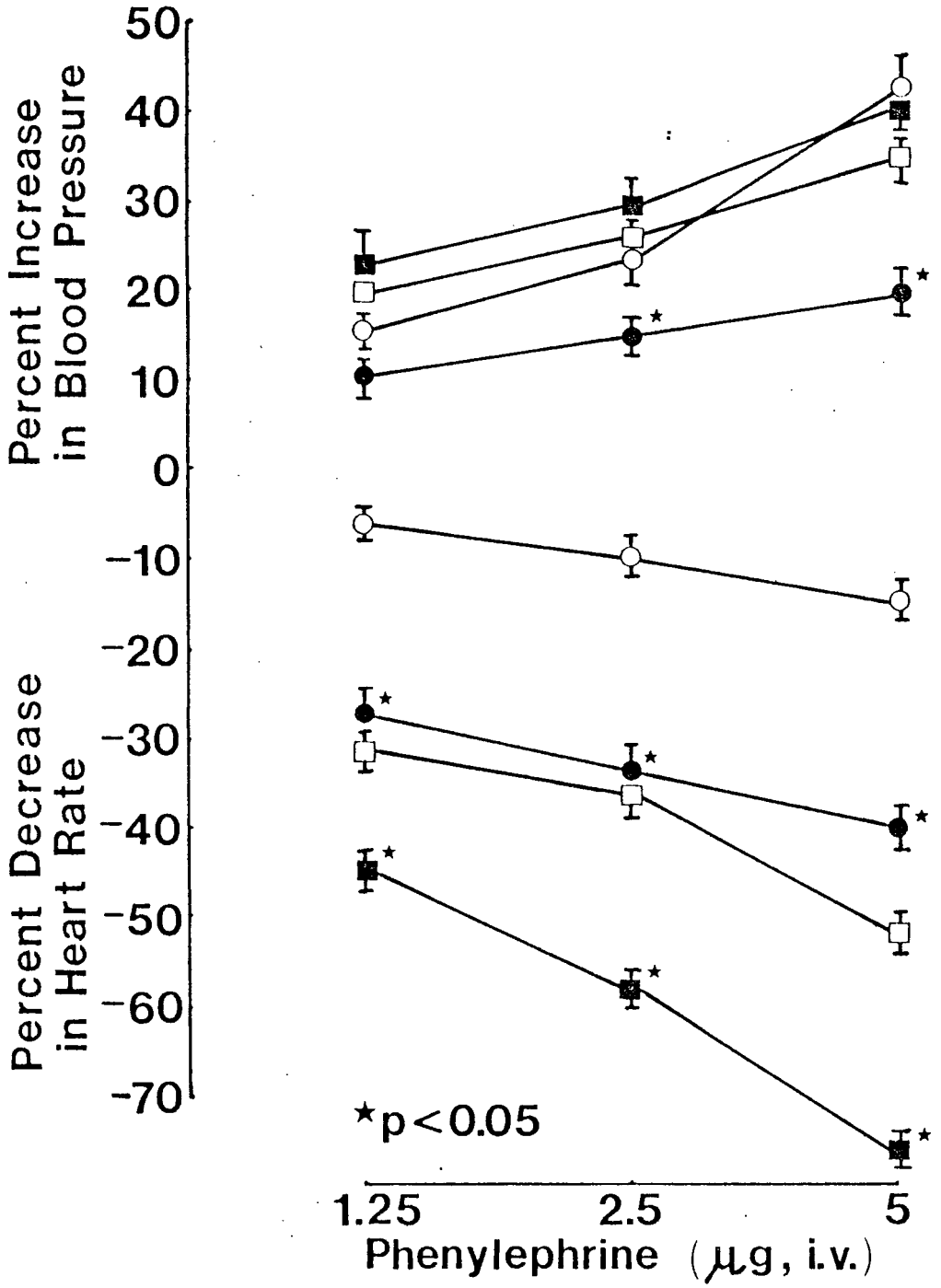
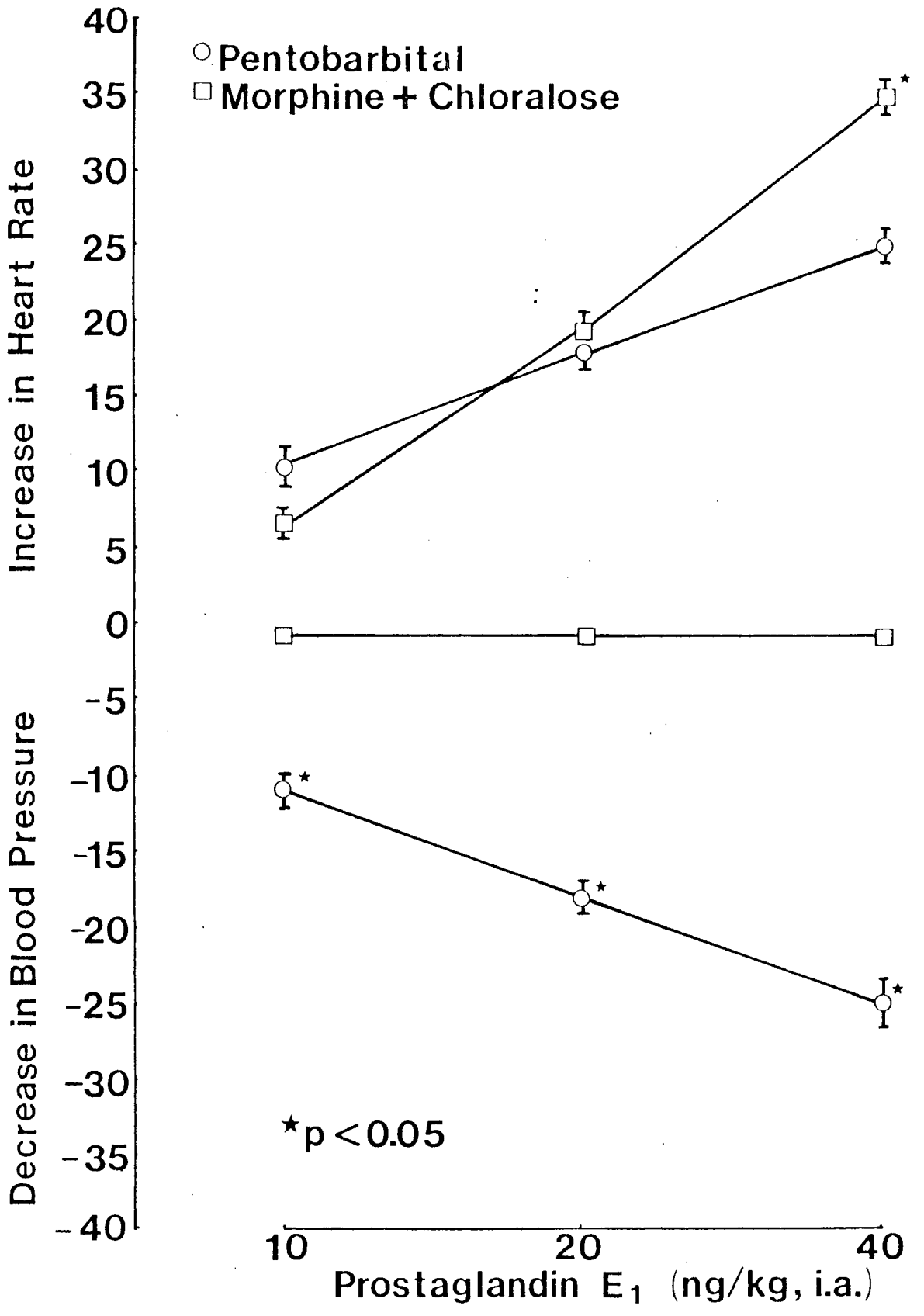

Percent Increase in Blood Pressure
Percent Decrease in Heart Rate
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
★p<0.05
1.25
2.5
5
Phenylephrine (μg, i.v.)

Figure 12. Increase in heart rate and decrease in blood pressure in response to prostaglandin E_1, 10, 20 and 40 ng/kg, i.a., in pentobarbital (o———o) and morphine-chloralose (□———□) anesthetized dogs. (N = 5 each group)


Pentobarbital
Morphine + Chloralose
Increase in Heart Rate
Decrease in Blood Pressure
40
35
30
25
20
15
10
5
0
-5
-10
-15
-20
-25
-30
-35
-40
★p < 0.05
10
20
40
Prostaglandin E1 (ng/kg, i.a.)

an increase in heart rate in response to PGE_1 administered i.a. The increase in heart rate was significantly higher in dogs anesthetized with morphine-chloralose at the higher dose compared to pentobarbital anesthetized dogs.

3.5 The effect of pentobarbital and morphine-chloralose anesthetia on the function of the carotid sinus baroreceptors

The effect of pentobarbital and morphine-chloralose anesthesia on the function of the carotid sinus baroreceptors was studied by stimulating the sinus nerve in dogs (at supramaximal voltage, 0.5 msec duration, and 2-32 pulses/sec frequency).

Figure 13 shows the percent changes in blood pressure and heart rate produced by carotid sinus nerve stimulation in dogs under pentobarbital and morphine-chloralose anesthesia. The percent decrease in blood pressure was of similar magnitude under both anesthetics, except at the highest frequency used (32 pulses/sec), where the percent decrease in blood pressure was significantly greater in the morphine-chloralose anesthetized dogs. Further, the percent decrease in heart rate was significantly greater in dogs anesthetized with morphine-chloralose at all levels of stimulation.

3.6 Effect of Δ^9-THC (2.5 mg/kg, i.v.) on systemic and pulmonary hemodynamics

The effect of Δ^9-THC (2.5 mg/kg, i.v.) on systemic and pulmonary hemodynamics was studied over a 90 minute period in dogs anesthetized with morphine-chloralose anesthesia.

Table 1 shows changes in blood pressure, heart rate, mean pulmonary arterial pressure, cardiac output, right ventricular stroke work, pulmonary

Figure 13. Percent decrease in blood pressure and heart rate in response to carotid sinus nerve stimulation at supramaximal voltage in pentobarbital (o———o) and morphine-chloralose (□———□) anesthetized dogs.

Pentobarbital: Resting MBP = 146.6 ± 4.5 mm Hg

Resting HR = 181.2 ± 5.5 beats/min (N = 5)

Morphine-chloralose: Resting MBP = 123.1 ± 8.1 mm Hg

Resting HR = 68 ± 3.2 beats/min (N = 6)

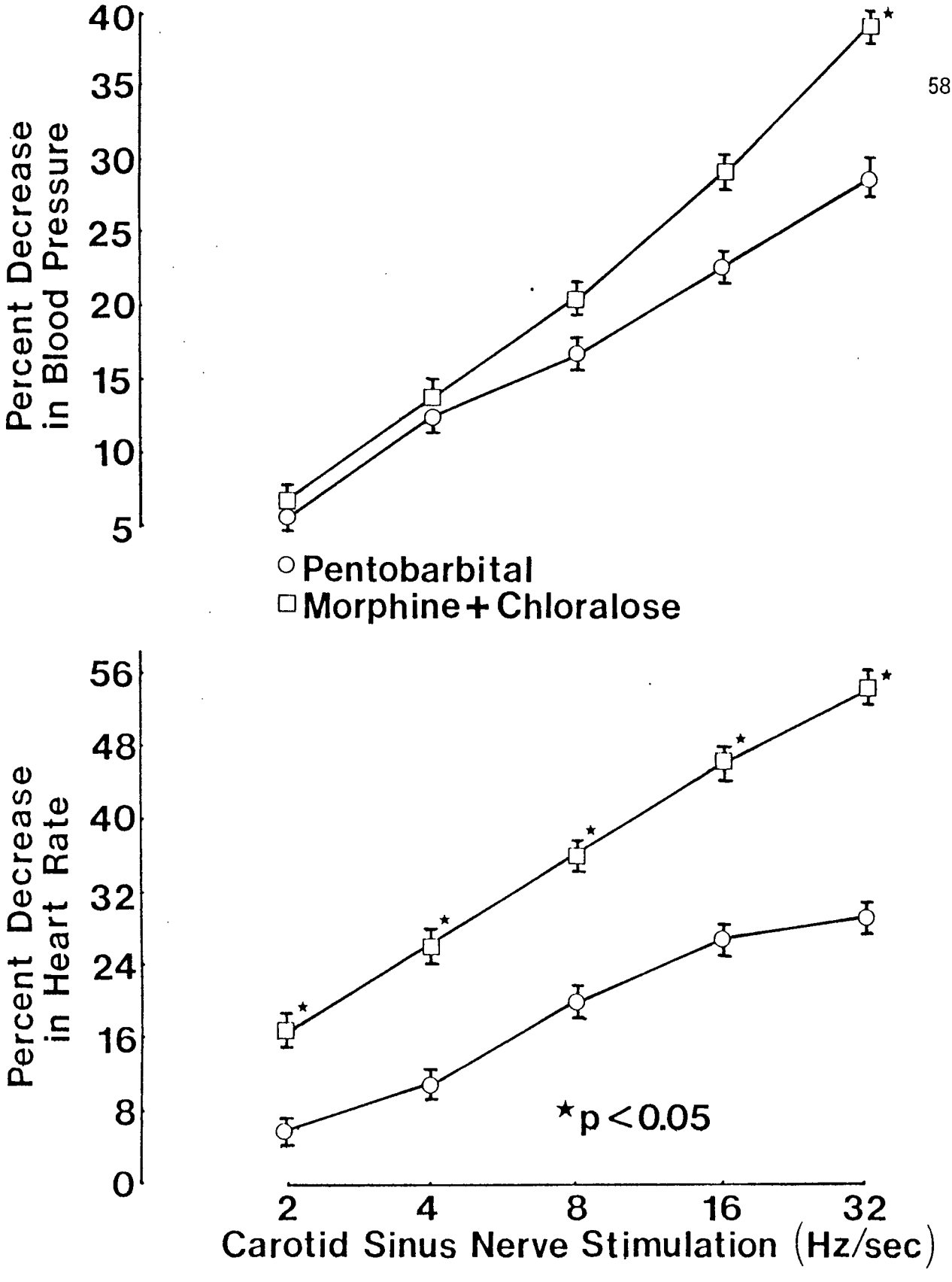

Percent Decrease in Blood Pressure
40
35
30
25
20
15
10
5
○ Pentobarbital
□ Morphine + Chloralose
Percent Decrease in Heart Rate
56
48
40
32
24
16
8
0
★p < 0.05
2
4
8
16
32
Carotid Sinus Nerve Stimulation (Hz/sec)

Table 1

Percent changes in various hemodynamic parameters after 5, 15, 30, 60 and 90 minutes of i.v. Δ^9-THC in morphine-chloralose anesthetized dogs.

Variable	Resting Value	5	15	30	60	90	Units
MBP	90.4 ± 6.0	↓20.1	↓23.9	↓24.1	↓21.4	↓16.6	mm Hg
HR	63 ± 2.0	↑26.3	↑29.8	↑25.2	↑25.8	↑22.2	beats/min
MPAP	16.4 ± 1.0	↓12.2	↓28	↓28	↓28	↓25.6	mm Hg
C.O.	1218 ± 144	↑24.4	↑ 8.5	↑ 8.8	↑ 7.4	↑ 5.4	ml/min
R.V.S.W.	3.4 ± .3	↓18.3	↓38.9	↓22.6	↓23.5	↓195	gram meter
R.V.R.	701 ± 115	↓23.5	↓41.5	↓42.8	↓40.8	↓34.8	dynes-sec/cm^5
T.P.R.	6358 ± 920	↓27.7	↓42.1	↓33.3	↓29.2	↓29.4	dynes-sec/cm^5

↑ = increase ↓ = decrease

MBP: mean blood pressure
HR: heart rate
MPAP: mean pulmonary arterial pressure
R.V.S.W.: right ventricular stroke work
P.V.R.: pulmonry vascular resistance
T.P.R.: total peripheral resistance

vascular resistance and total peripheral resistance over a 90 minute period after the administration of 2.5 mg/kg of Δ^9-THC. Heart rate and cardiac output showed a maximum increase of 29.8 and 28%, respectively. While the mean blood pressure, pulmonary arterial pressure, right ventricular stroke work, pulmonary and total vascular resistance all showed a decrease ranging from 24-42%, the changes reached a maximum between 5-30 minutes after the administration of the drug.

3.7 Effect of anesthetics and SQ-14,225 on plasma renin activity

Pentobarbital and morphine-chloralose anesthesia produced a significant increase in the plasma renin activity at 15, 30 and 60 minutes after their administration to mongrel dogs (Figure 14). The level of the plasma renin activity continued to increase over the sixty minutes measurement period, and the values were significantly greater in dogs anesthetized with morphine-chloralose at 30 and 60 minutes when compared to the pentobarbital group (Figure 14). Administration of SQ-14,225 caused a significant increase in the plasma renin activity in both groups of anesthetized dogs at 15, 30 and 60 minutes after the drug administration (Figure 15). However, the increase in the plasma renin activity was greater in the animals anesthetized with pentobarbital than in those that received morphine-chloralose anesthesia (Figure 15). SQ-14,225 produced similar decreases in blood pressure in both groups of anesthetized dogs. However, the heart rate of the morphine-chloralose animals showed an increase of 22 beats/min at 15 minutes after the drug administration, which was followed by partial recovery (Figure 16). Dogs anesthesized with pentobarbital showed a slight decrease in their heart rate in response to the drug (Figure 16).

Figure 14. Represents the plasma renin activity (ng of AngI/ml/hr) in conscious and 15, 30 and 60 minutes after the administration of pentobarbital (▨) and morphine-chloralose (□) anesthesia.

Pentobarbital: Resting BP = 132 mm Hg

Resting HR = 200 beats/min

Morphine-chloralose: Resting BP = 95 mm Hg

Resting HR = 80 beats/min

($P^* < 0.05$), N = 5 dogs in each group

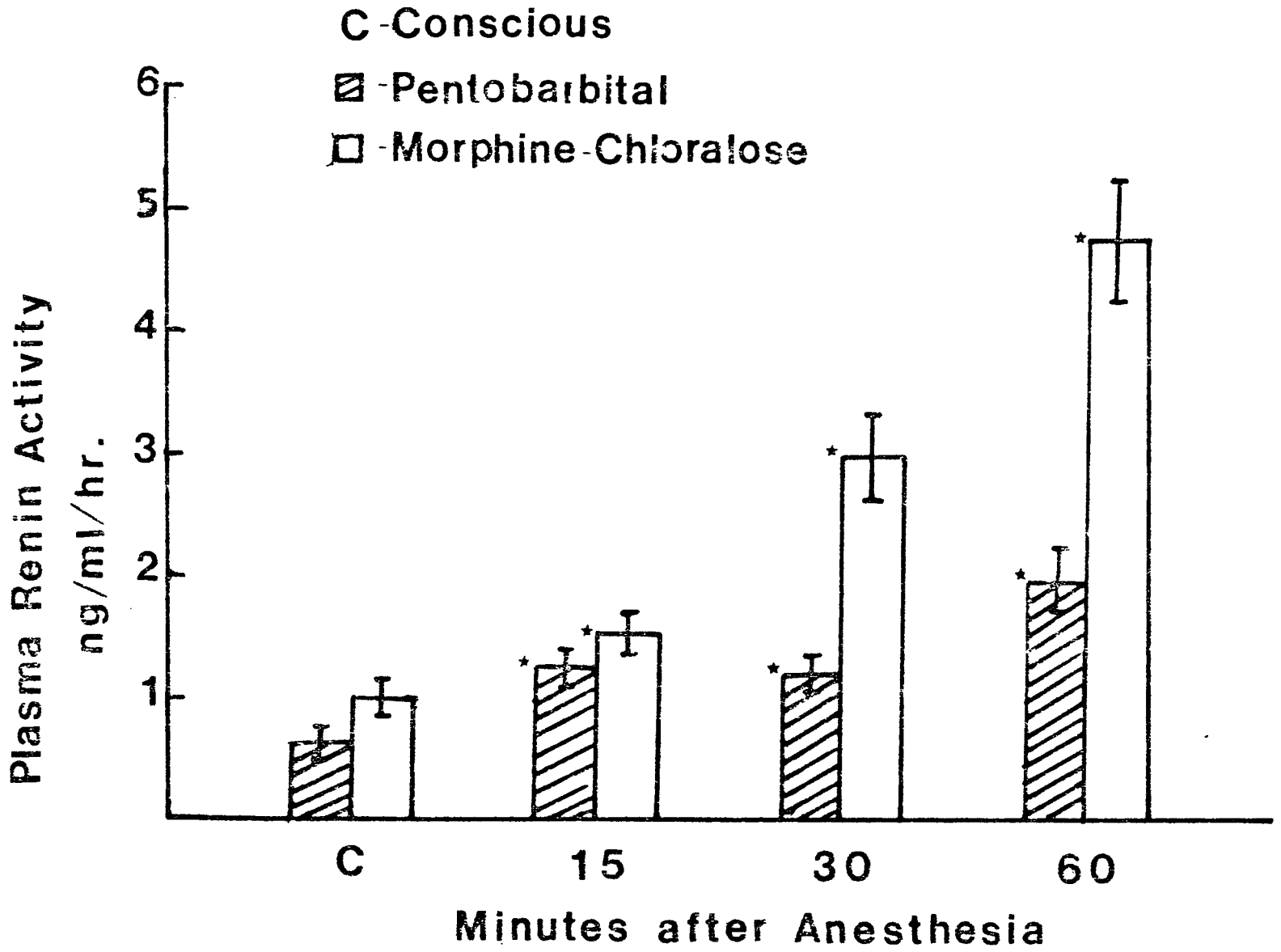

C-Conscious
-Pentobarbital
-Morphine-Chloralose
Plasma Renin Activity
ng/ml/hr.
6
5
4
3
2
1
C
15
30
60
Minutes after Anesthesia

Figure 15. Shows the plasma renin activity (ng of AngI/ml/hr) before, 15, 30 and 60 minutes after the administration of SQ-14,225 (3 mg/kg, i.v.) to pentobarbital (▨) and morphine-chloralose (□) anesthetized dogs.

($P^* < 0.05$), N = 5 dogs in each group

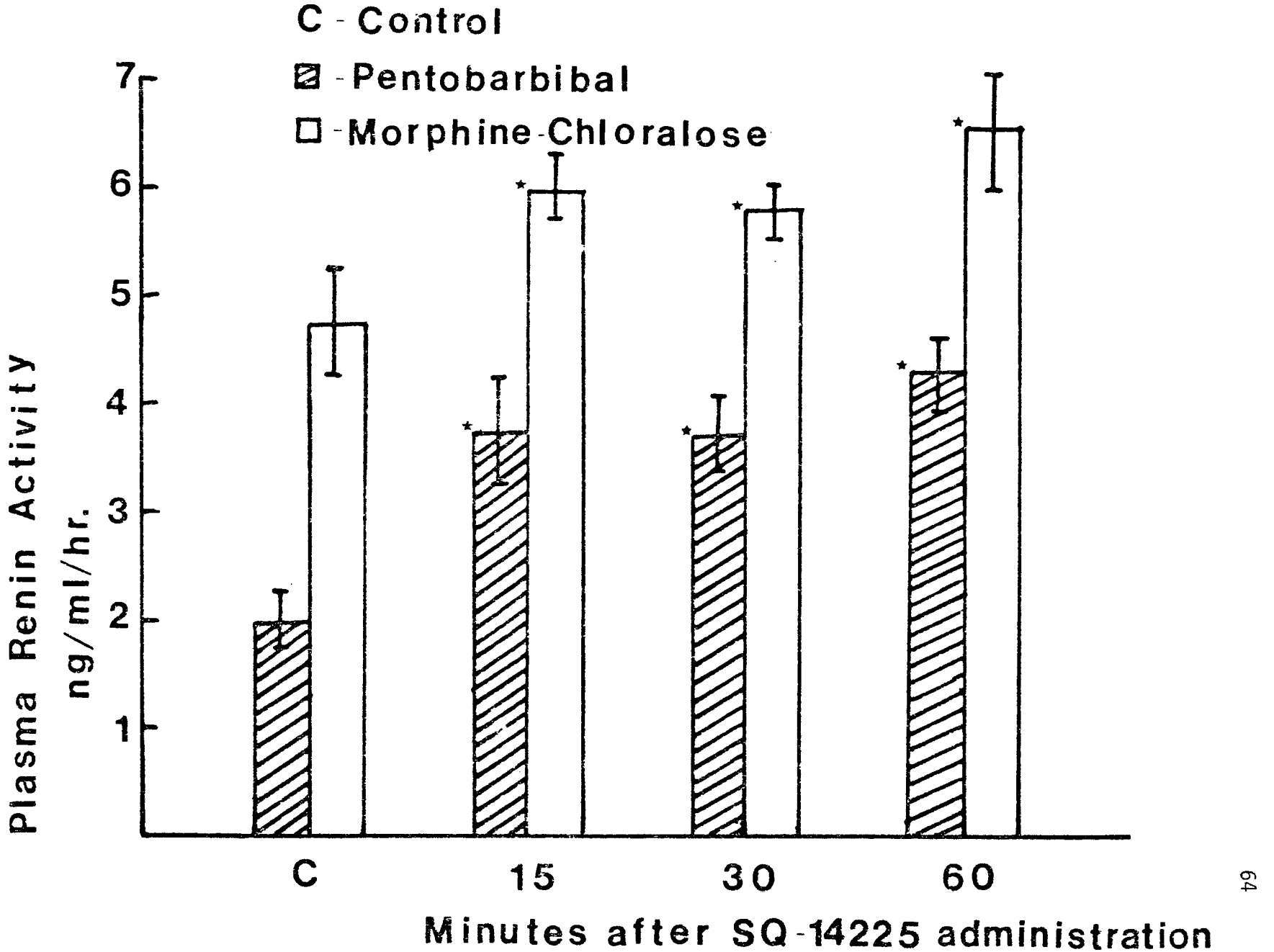
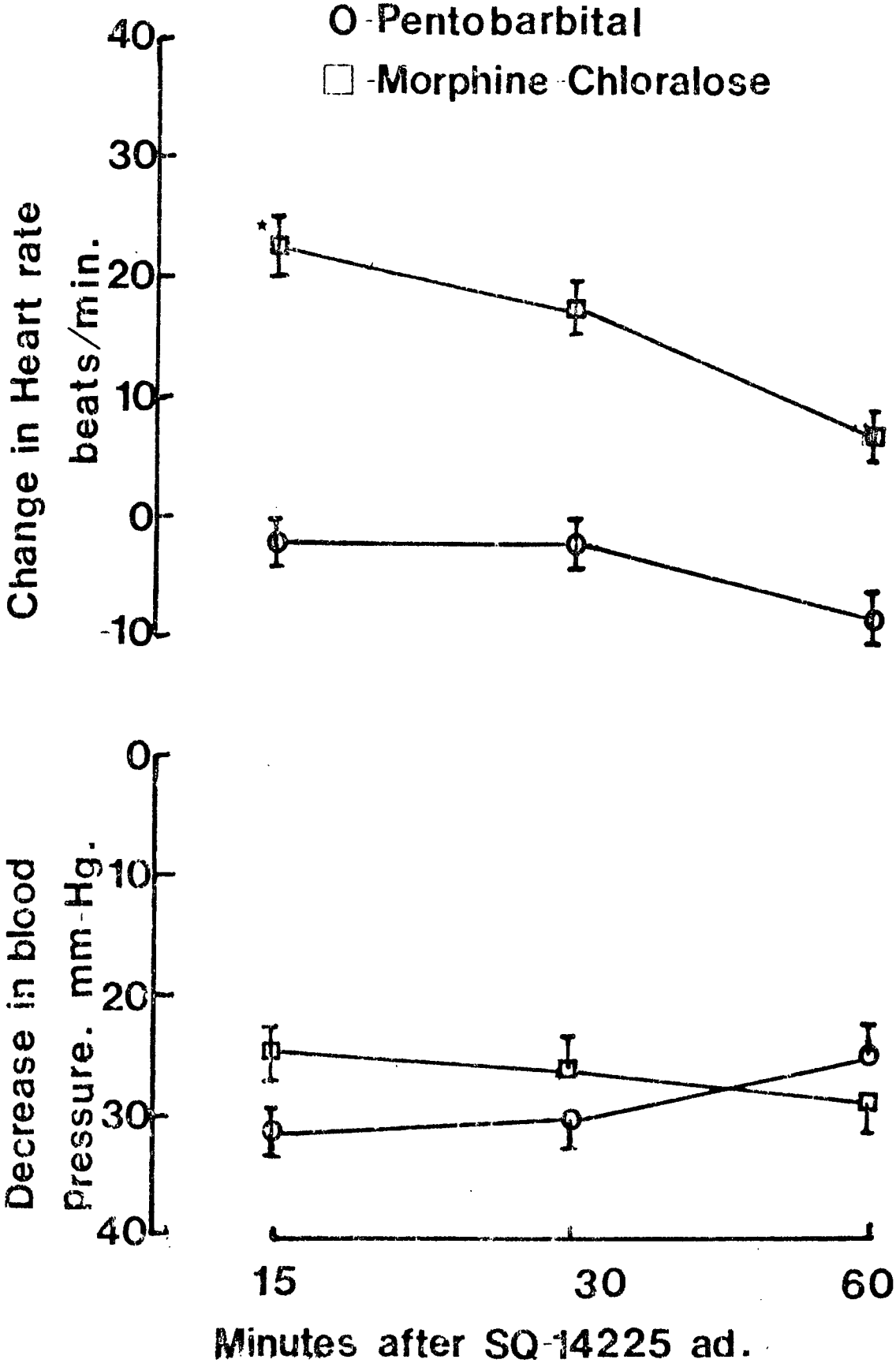

C - Control
- Pentobarbibal
- Morphine-Chloralose
Plasma Renin Activity
ng/ml/hr.
7
6
5
4
3
2
1
C
15
30
60
Minutes after SQ-14225 administration

Figure 16. Represents the decrease in blood pressure and change in heart rate at 15, 30 and 60 minutes after the administration of SQ-14,225 to pentobarbital (o———o) and morphine-chloralose (□———□) anesthetized dogs.

($P^* < 0.05$), N = 5 dogs in each group


O-Pentobarbital
□-Morphine-Chloralose
Change in Heart rate beats/min.
40
30
20
10
0
-10
Decrease in blood Pressure. mm-Hg.
0
10
20
30
40
15
30
60
Minutes after SQ-14225 ad.

VI. Discussion:

The purpose of this investigation has been to identify the differences in the resting cardiovascular parameters of the mongrel dogs under the influence of the two anesthetic agents studied and to evaluate how these two anesthetics may influence the physiological effects of certain pharmacological agents.

Previously investigators have reported the influence of these and other anesthetics on certain hemodynamic parameters such as cardiac output, heart rate, stroke volume, etc. (21,26,28,33,44). However, only a limited amount of information is available on the neurogenic control of peripheral vasculature and on the vascular smooth muscle.

Pressure flow curves obtained from perfused hind limb studies are very useful in determining resting neurogenic tone and other factors involved in the control of peripheral vascular resitance (69,70). In the present studies, the magnitude of the shifts of the pressure flow curves to the right following acute denervation and after alpha-adrenergic blockade are essentially identical in the groups of dogs anesthetized with sodium pentobarbital or with the combination of morphine plus chloralose (M + C). Since the peripheral sympathetic transmission to the hind limb vasculature appeared to be depressed in M + C group in comparison with the pentobarbital group, it is possible that in M + C group increased sympathetic discharge from the central nervous system, together with enhanced responsiveness of the vasculature to norepinephrine may be responsible for lack of differences in the resting tone.

The results do indicate such vasoconstrictor responses to intra-arterial norepinephrine were significantly greater in M + C group. It is also possible that the higher levels of plasma renin activity under M + C anesthesia may also have contributed to the maintenance of resting neurogenic tone since it has been demonstrated that angiotensin II could enhance norepinephrine release from sympathetic nerve endings (71). It may also be suggested that peripheral sympathetic transmission appeared to have been enhanced under barbiturate anesthesia because of the inhibition of cholinergic vasodilator fibers activity during electrical stimulation under this anesthesia (48). Such a possibility cannot be excluded since Jandhyala et al. (72) have previously demonstrated that the responsiveness of the cardiac sympathetic nerves was identical in dogs under these two anesthetics. Brezenhoff (54) also showed that under chloralose anesthesia, vascular responses to norepinephrine were significantly greater than those obtained under pentobarbital in rats. In contrast, in the present study, pressor responses to biogenic amines mediated by action of these agents on alpha-adrenergic receptor activity of the beta adrenergic and muscurinic receptors was similar in both the groups.

Pressor responses in M + C anesthetized animals did not result in increased tone contributed by circulating catecholamines. Thus, it may be suggested that the level of circulating catecholamines are lower in the M + C group in comparison with the barbiturate group. One significant finding from these perfused hind limb studies is that the

intrinsic tone of the vascular smooth muscle was significantly higher in M + C dogs than in those anesthetized with sodium pentobarbital. Such a reduction in the vascular tone may have reflexly contributed to the marked increase in the heart rate consistently noted after pentobarbital administration. Such an increase in the heart rate has been reported to be due to inhibition of central vagal tone by pentobarbital anesthesia (22,23). However, this inhibition may have been mediated via a reflex mechanism). The studies of Manders et al., (34) also support this conclusion.

Several investigators studied the influence of anesthetic agents on reflexly mediated responses of the cardiovascular system (54,55,56). In general, most studies seem to indicate that the cardiovascular reflexes are most active under chloralose anesthesia (54,60,61). Addition of morphine increases muscle relaxation necessary for surgical procedures. However, it is not clear how the addition of morphine would affect cardiovascular reflexes. In the present studies, systemic pressor responses to bilateral carotid occlusion (BCO) were identical in both the groups; however, hind limb vasoconstrictor responses to BCO (as indicated by the increase in the perfusion pressure) were significantly greater in the M + C anesthetized dogs in comparison with the pentobarbital group. The data may suggest that regional resistances are not uniformly affected by BCO. It is also possible that an increase in the alpha-adrenergic receptor activity in the hind limb (as reported earlier) may have contributed to this greater increase in the perfusion pressure in the M + C group. Similarly, the reflex vasodilator responses to intra-

venous norepinephrine were significantly greater in the M + C group. However, it should be noted that an attenuation of cholinergic vasodilator activity by pentobarbital (48) may also have contributed to the reduction in reflex vasodilation. Thus, from hind limb studies it was not conclusive how the cardiovascular reflexes were influenced by these two anesthetic agents.

The magnitude of the blood pressure responses to intravenous norepinephrine varied; consequently, it is not possible to conclude how the two anesthetics influenced these pressor responses. However, irrespective of the magnitude of the pressor responses, reflex bradycardic responses were always significantly greater in the M + C group. Phenylephrine produced identical changes in the blood pressure in the M + C group and pentobarbital dogs; however, reflex bradycardic responses were markedly and significantly greater in the M + C group. The differences in the increase in blood pressure and decrease in heart rate in response to norepinephrine and phenylephrine could be due to: a) the ability of norepinephrine to act on B_1 receptors on the heart, b) different alpha-receptors which norepinephrine and phenylephrine act on has been proposed recently by Bently et al., (65), or c) a combination of the above two possibilities. Carotid sinus nerve stimulation studies further confirmed that the cardiovascular reflexes may be potentiated in M + C anesthetized dogs; decrease in the heart rate to sinus nerve stimulation was significantly greater at all the frequencies of stimulation (2 to 32 Hz). In contrast, depressor responses to sinus nerve stimulation were significantly greater in the M + C group only at the highest frequency used. Since vagal activation plays a prominent role

responses to norepinephrine were also significantly greater in this group.

Effect of Anesthetics on Drug Responses

On clonidine

Intravenous administration of clonidine has been shown to cause increased vagal reflex bradycardia in dogs, elicited by pressor drugs such as norepinephrine and angiotensin (73). Similar results were also obtained by Nayler (74). Kobinger and Walland (75) have shown that the facilitation of the vagal bradycardic reflex produced by clonidine (i.v., i.c.i.) in conscious and pentobarbital anesthetized dogs is due to its action on the central alpha-receptors. The present study does not deal with the mechanism of action of clonidine, but whether clonidine produces this bradycardic facilatory effect in response to i.v. norepinephrine and phenylephrine in dogs under pentobarbital and M + C anesthesia. The increases in the blood pressure to norepinephrine were similar in both groups before and after clonidine. However, the reflex decrease in the heart rate to norepinephrine was facilitated significantly by clonidine only in the animals anesthetized with pentobarbital, but not in those under M + C. In contrast, phenylephrine induced reflex bradycardia was potentiated by clonidine under both anesthetics. The explanation of this could be due to the ability of pentobarbital to depress the S-A node as shown by Cox (33), which may suggest that the effect of the administered norepinephrine on the heart was minimized and so was its interference with the facilitated bradycardia produced by clonidine. This depression of the S-A node has not been shown to occur in the animals anesthetized with morphine-

chloralose. Thus the activity of norepinephrine on the B_1 receptors could interfere with the facilitated bradycardia produced by clonidine in M + C animals.

Since phenylephrine has no such agonistic activity on cardiac receptors, clonidine induced potentiation of the reflex bradycardia occurred in both the groups.

On prostaglandin E_1 (PGE_1)

Intravenous administration of PGE_1 in 10, 20 and 40 ng/kg doses produced no significant change in blood pressure or heart rate in dogs anesthetized with pentobarbital or M + C. This could be due to the presence of prostaglandin metabolizing enzymes in the pulmonary circulation (76), resulting in rapid destruction of the drug and its inability to reach the systemic circulation.

Intra-aortic (ascending) administration in the same doses of PGE_1 caused a very marked decrease in blood pressure and a tachycardia in the dogs anesthetized with pentobarbital, while these same doses of PGE_1 produced no change in blood pressure of dogs anesthetized with morphine-chlorose. Similar increases in the heart rate occurred at 10 and 20 ng/kg doses and a significantly greated increase at 40 ng/kg, when compared with the pentobarbital animals.

Since PGE_1 is considered to be one of the most potent vasodilator substances known (77), the increase in the heart rate in the pentobarbital dogs may be due to reflexogenic mechanisms triggered in response to the decrease in blood pressure. However, the increase in heart rate in the M + C group may be due to a different yet to be identified mechanism, since PGE_1 failed to reduce blood pressure.

The mechanism involved in these observations was not clear and deserves further investigation.

On Δ^9-tetrahydrocannabinol (Δ^9-THC)

Systemic and pulmonary hemodynamic effects of this agent have been extensively reported in pentobarbital anesthetized dogs. Thus, the present studies were conducted using the same dose only in the M + C anesthetized animals.

Intravenous administration of Δ^9-THC (2.5 mg/kg) to morphine-chloralose anesthetized dogs caused a significant increases in the heart rate, cardiac output, and decreases in systemic arterial pressure, pulmonary arterial pressure, right ventricular stroke work, pulmonary vascular resistance and total peripheral resistance. All these changes occurred 5-30 minutes after the administration.

Previous studies conducted in this department (78-80) demonstrated that in pentobarbital anesthetized dogs using similar doses of THC produced significant and consistent reduction in heart rate, cardiac output, and arterial blood pressure, while the change in total peripheral resistance was variable. Further, in pentobarbital anesthetized dogs, THC increased pulmonary artery pressure, pulmonary vascular resistance and right ventricular stroke work (78,79,80). Thus, except for the hypotensive action, pulmonary and systemic hemodynamic effects of this agent were completely opposite to those noted in the M + C group. These studies indicate complex interaction and influence of anesthetics on the effects of Δ^9-THC. It is not clear how bradycardic effects of THC were reversed by M + C anesthesia. The complex nature of this interaction was further emphasized by the findings of Jandhyala and Buckley

(65) that pretreatment with morphine or chloralose alone is not sufficient to demonstrate tachycardia to THC. Simultaneous presence of both these agents is essential to produce this effect.

The increase in heart rate produced by THC in the morphine-chloralose animals is similar to that produced in humans, as shown by Weiss et al., (81). The decreased pulmonary vascular resistance is quite similar to the decrease in airway resistance in man following THC administration as described by Vachon et al., (82) and Tashkin et al., (83). The data suggest that M + C anesthesia is perhaps more useful in investigating the effects of cannabinols in dogs, since these actions may be similar to that in man.

On Plasma renin activity (PRA) and on angiotensin I converting enzyme (ACE) inhibitor, SQ-14,225

Pentobarbital anesthesia has been shown to increase the level of the PRA in mongrel dogs by Johnson et al., (40) and Lokhandwala et al., (41). However, the effect of morphine-chloralose anesthesia on the PRA is still unclear. The present study concerns itself with the ability of pentobarbital and morphine-chloralose anesthesia to change the level of the PRA in dogs and to study how these affect the activity of SQ 14,225 (D-3-mercapto-2-methylpropanoyl-L-proline).

Both pentobarbital and morphine-chloralose caused a significant and progressive increase in the plasma renin activity at 15, 30 and 60 minutes after their administration. The increase in PRA was twice as much at 30 and 60 minutes in the group anesthetized with morphine-chloralose when compared with the other group. The gradual increase in the PRA over the

60 minute period may be due to time dependent alterations in the autonomic activity or in the hemodynamic parameters such as cardiac output, stroke volume and total peripheral resistance produced by anesthetics (26,28).

SQ-14,225 has been shown to be a specific inhibitor in vivo and in vitro of the angiotensin I converting enzyme in a number of species (84,85). Intravenous administration of this compound to dogs anesthetized with pentobarbital and morphine-chloralose caused a decrease in mean blood pressure ranging from 25-32 mm Hg in both groups of anesthetized animals. Whereas the heart rate of morphine-chloralose dogs showed a significant increase at 15 minutes after the administration of SQ-14,225, the dogs under pentobarbital showed a slight decrease in heart rate. This could be due to the inability of the baroreceptor reflex mechanism to respond to changes in blood pressure in the pentobarbital anesthetized animals and/or it could be due to the ability of pentobarbital to depress the heart. SQ-14,225 also caused a significant increase in the PRA in both groups of the anesthetized dogs at 15, 30 and 60 minutes after its administration. However, the increase in PRA was much larger in dogs anesthetized with pentobarbital than in those anesthetized with morphine-chloralose.

Despite the differences in the predrug PRA levels, hypotension produced by SQ-14,225 was equal in magnitude in M + C and pentobarbital groups, indicating that the hypotensive activity of this agent may be to a degree independent of the PRA levels in the animals.

The results of this investigation demonstrated that the anesthetics studied possess multiple and diverse actions on the physiological systems.

Further, these actions are not restricted to the central nervous system, and independent of their ability to induce anesthesia, the peripheral actions of these two anesthetics appear to include effects on vasculature, on the autonomic receptor and on peripheral sympathetic transmission. The mechanisms involved in these alteration deserves further investigation since these actions of anesthetics may have influence on the mechanisms of action of other agents. A recent report by Neilsen (86) clearly demonstrated how anesthetic influence could obscure or invalidate the results obtained with the drugs acting on the peripheral sympathetic nervous system. In the present studies involving clonidine, Δ^9-tetrahydrocannabinol and prostaglandins, further confirm the view that the anesthetic influence cannot be minimized while interpreting the mechanism of action of pharmacological agents. The results of this study emphasize the importance of systematically establishing the physiological effects of various anesthetics used in preclinical studies. Such information will be most useful for scientists in the selection of a suitable anesthetic agent and in accurate interpretation of the data obtained.

CONCLUSIONS

The following conclusions can be drawn from this investigation:

1. The neurogenic tone and the tone contributed by circulating catecholamines in the hind leg is similar in both groups of animals; however, pentobarbital seems to depress the intrinsic tone of the smooth muscle.

2. The alpha-receptors of the hind limb are more responsive to norepinephrine in the morphine-chloralose dogs, while the B_2 and muscarinic receptors seem to have the same responsiveness to their respective agonists.

3. Autonomic reflexes appear to be more active to changes in blood pressure in the morphine-chloralose animals.

4. Clonidine caused significant potentiation in the bradycardic response to norepinephrine pressor response in the pentobarbital dogs, but caused significantly potentiated bradycardic response to phenylephrine in both groups of anesthetized animals.

5. Dogs anesthetized with morphine-chloralose seem to respond differently to pharmacological agents such as prostaglandin E_1, Δ^9-tetrahydrocannabinol and SQ-14,225.

6. Both pentobarbital and morphine-chloralose caused a significant increase in the plasma renin activity of mongrel dogs; however, they have no influence on the magnitude of the hypotensive response of SQ-14,225.

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