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MECHANISMS FOR THE IN VIVO CARDIOVASCULAR ACTIONS OF 1-alpha-ACETYLMETHADOL

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Douglas Charles Eikenburg

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Ph.D. degree in Pharmacology/Toxicology

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MECHANISMS FOR THE IN VIVO CARDIOVASCULAR ACTIONS OF l-alpha-ACETYLMETHADOL

By

Douglas Charles Eikenburg

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
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ABSTRACT

Mechanisms for the In Vivo Cardiovascular Actions of
l-alpha-Acetylmethadol

by

Douglas Charles Eikenburg

The purpose of this investigation was to examine, and determine mechanisms for the in vivo cardiovascular effects of l-alpha-acetylmethadol (LAAM) and its two active metabolites, l-alpha-acetylnormethadol (nor-LAAM) and l-alpha-acetyldinormethadol (dinor-LAAM). LAAM and its metabolites decreased mean arterial blood pressure (BP), heart rate (HR) and contractile force (CF) when given intravenously to anesthetized dogs. The minimum doses of LAAM, nor-LAAM, and dinor-LAAM required to significantly decrease BP and HR were 1.4, 0.13, and 1.3 mg/kg, respectively. The minimum dose of each drug required to significantly decrease CF was approximately the same, 1.4 mg/kg. The BP response to LAAM appeared to be the result of effects on both the cardiac output and peripheral resistance.

The cardiovascular response to LAAM in vagotomized animals was not significantly different from that observed in intact animals. Atropine, a muscarinic cholinergic receptor antagonist, attenuated the HR response to LAAM. The possibility that LAAM inhibits cholinesterase and thereby produces part of its negative chronotropic effect was examined. LAAM and its metabolites inhibited purified preparations of acetylcholinesterase and butyrylcholinesterase. These drugs decrease the rate of

enzymatic hydrolysis of acetylcholine by guinea pig heart homogenates and guinea pig plasma. However, comparison of the anticholinesterase and negative chronotropic actions suggests that cholinesterase inhibition is not the primary mechanism for the negative chronotropic effects of LAAM which are antagonized by atropine.

In chemically sympathectomized animals, the minimum dose of LAAM required to significantly decrease BP was greater than in intact animals. The magnitudes of the BP and HR responses to LAAM in chemically sympathectomized animals were decreased when compared with the results in intact animals. The data suggest that LAAM decreases BP and HR, in part, by an action on the sympathetic nervous system. Chemical sympathectomy affected neither the magnitude of the CF response to LAAM nor the minimum dose of LAAM required to produce the response.

LAAM decreased BP, HR, and the responses of the nictitating membranes to sympathetic nerve stimulation in the cat. The minimum doses of LAAM required to produce these effects were identical, 1.4 mg/kg. Naltrexone pretreatment, 3 mg/kg s.c., completely blocked the effect of LAAM on BP and partially blocked the effect of LAAM on HR. Naltrexone completely antagonized the effects of LAAM on the nictitating membrane responses to nerve stimulation. The data suggest that LAAM interacts with opiate binding sites to produce its effects on BP, perhaps by an action on the sympathetic nervous system. A similar mechanism appears to contribute to the effect of LAAM on HR. The interaction of LAAM with opiate binding sites also appears to contribute to the effect of LAAM on CF.

LAAM caused a significant decrease in BP, HR and CF in dogs which had been both chemically sympathectomized and vagotomized. These data

suggest that cardiac effects, independent of neural influences, contribute to the cardiovascular response to LAAM.

In summary, LAAM and its major metabolites nor-LAAM and dinor-LAAM have significant depressant effects on the cardiovascular system. These effects appear to be the result of an action to depress sympathetic nervous system function and other actions on the heart not dependent on neural influences.

to Barb whose love, understanding, and encouragement made this possible and to my family who have been behind me from the beginning of this endeavor

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INTRODUCTION

A. General Background

l-alpha-Acetylmethadol, more commonly referred to as LAAM, is a long-acting, orally effective narcotic agonist. Study of the pharmacology and toxicology of LAAM dates back to the late 1940's and early 1950's. Merck and Company first investigated LAAM as a possible long-acting, orally effective analgesic to be used in surgical patients. The analgesic action of LAAM was of long onset, short duration and required large doses. Furthermore, coma was observed in some patients who had received successive doses of LAAM (Keats and Beecher, 1952). Although these patients also had received morphine at the same time, the investigators believed there was a connection between LAAM and the comas. Therefore, Merck and Company stopped the investigation. Later studies determined that these early problems in all likelihood were related to the presence of two major active metabolites of LAAM, l-alpha-acetylnormethadol (nor-LAAM) and l-alpha-acetyldinormethadol (dinor-LAAM), both of which have long plasma half-lives (Sung and Way, 1954; Veatch et al., 1964; McMahon et al., 1965).

Also in the early 1950's, Fraser and Isbell found that LAAM could relieve and prevent the symptoms of the abstinence syndrome in heroin addicts for up to 72 hours after a single oral dose (Fraser and Isbell, 1951). This long duration of action and the oral effectiveness of LAAM led to the suggestion that LAAM be studied as a substitute for methadone

in heroin addict treatment (Jaffe et al., 1970). In 1971, an executive order established the Special Action Office for Drug Abuse Prevention (SAODAP). Its objective was to develop a comprehensive strategy to combat drug abuse. A first step in this process was an extensive review of the literature pertaining to heroin addiction treatment. It was concluded that LAAM was the most promising long-acting agent which could block withdrawal symptoms. Therefore, SAODAP began the development of LAAM for this use. SAODAP has been disbanded since that time and the development of LAAM currently is the responsibility of the National Institute for Drug Abuse (NIDA) (Blaine and Renault, 1976a).

At the present time, LAAM is in phase III clinical trials. However, little information regarding the pharmacology and toxicology of LAAM is available, an unusual situation considering the advanced stage of the development of LAAM. Several of the early studies examining the toxicology of LAAM in animals have been invalidated and little pharmacological and toxicological information about LAAM can be found in peer-reviewed literature. Government publications such as proceedings of the Committee on Problems of Drug Dependence and National Institute of Drug Abuse (NIDA) monographs which contain information pertaining to the use of LAAM indicate to me a deficiency of information about the pharmacology and toxicology of LAAM. This is particularly true with regard to the effects of LAAM, or lack thereof, on the cardiovascular system. These effects have been investigated only recently, perhaps because narcotics as a drug class are considered to have little if any direct cardiovascular action (Jaffe and Martin, 1975).

B. Cardiovascular Actions of l-alpha-Acetylmethadol and Its Metabolites

The actions of the metabolites nor-LAAM and dinor-LAAM contribute to the long duration of action of LAAM (Sung and Way, 1954; Veatch et al., 1964; McMahon et al., 1965). Nor-LAAM and dinor-LAAM have long plasma half-lives and cumulate in the plasma of patients chronically receiving LAAM (Billings et al., 1974; Kaiko and Inturrisi, 1975; Henderson et al., 1977a). For this reason, the cardiovascular actions of nor-LAAM and dinor-LAAM, as well as LAAM, have been studied.

Stickney (1977a) compared the chronotropic effects of LAAM, nor-LAAM and dinor-LAAM to those of morphine and a structural analogue of LAAM, methadone in the isolated guinea pig right atrium preparation. LAAM produced significant negative chronotropic effects at concentrations of $5x10^{-6}$M or greater. Morphine at similar concentrations produced no significant effect. Methadone produced negative chronotropic effects, but was less potent than LAAM. The major metabolites of LAAM nor-LAAM and dinor-LAAM, also produced significant chronotropic effects. Nor-LAAM produced a biphasic response, causing a positive chronotropic response at concentrations up to $1x10^{-6}$M, and a negative chronotropic response at higher concentrations. The effects of dinor-LAAM were similar to those of LAAM. The chronotropic actions of LAAM and its metabolites were not antagonized by naloxone, indicating that these actions do not involve drug interactions with opiate binding sites. The concentrations of LAAM at which the negative chronotropic effects were observed are within an order of magnitude of LAAM plasma concentrations measured in patients chronically receiving LAAM (Billings et al., 1974; Kaiko and Inturrisi, 1975; Henderson et al., 1977a,b).

Subsequently, Stickney (1978a) examined the possible involvement of muscarinic cholinergic receptors in the negative chronotropic actions, and β-adrenergic receptors in the positive chronotropic actions of LAAM and its metabolites. The negative chronotropic actions of LAAM were attenuated, but not completely blocked, by atropine. LAAM enhanced the negative chronotropic activity of methacholine instead of methadone in a manner similar to physostigmine, a cholinesterase inhibitor. It was concluded that LAAM produced part of its negative chronotropic effect by activating muscarinic receptors, perhaps via cholinesterase inhibition. An action on the heart, not involving muscarinic receptors, also is involved in the negative chronotropic response to LAAM. No exact mechanism has been elucidated. Propranolol completely antagonized the positive chronotropic activity of nor-LAAM. This demonstrated that nor-LAAM produced the positive chronotropic response by activating β-adrenergic receptors.

Stickney (1977b) also has examined the effects of LAAM and its metabolites on cardiac mechanical function in the isolated guinea pig left atrium preparation. LAAM, nor-LAAM, and dinor-LAAM, at concentrations up to $1x10^{-5}$M, produced a positive inotropic response. A negative inotropic response was observed at higher concentrations. Naloxone pretreatment had no effect. LAAM and its metabolites do not appear to interact with opiate binding sites to produce their inotropic effects. In a later study, Stickney (1978b) examined the mechanisms for these inotropic effects. Propranolol totally blocked the positive inotropic response. Further investigation revealed that LAAM, nor-LAAM and dinor-LAAM activate β-adrenergic receptors by releasing tissue

catecholamines. The negative inotropic response to LAAM and its metabolites appeared to involve calcium antagonism, but the exact mechanism was not established.

Lee and Berkowitz (1977) have studied the effects of LAAM on blood vessels. Their report described the effects of LAAM and several structurally-related narcotics on the calcium-dependent contraction of isolated rat aortic smooth muscle strips. LAAM inhibited these contractions. This action of LAAM was overcome by increasing the calcium concentration in the extracellular medium. It was concluded that LAAM acted as a calcium antagonist. No significant effect on resting vessel tension was observed, however.

Reports describing the in vivo effects of LAAM on the cardiovascular system are scant. LAAM, at doses of 1.3 mg/kg i.v. or greater, decreases heart rate in anesthetized dogs (Stickney and Schwartz, 1977). This effect was partially antagonized by atropine. Waters and coworkers (1978) reported that an oral dose of 0.3 mg/kg LAAM significantly decreased heart rate in conscious dogs. LAAM also has been reported to have cardiodepressant effects when chronically administered p.o. to monkeys (Masten et al., 1978). Cardiovascular responses to LAAM have been reported in the clinical literature. For example, a study by Ling and coworkers (1976) revealed incidences of postural hypotension and pulse irregularities in patients chronically receiving LAAM. Unfortunately, a summary of LAAM clinical literature by Blaine and Renault (1976b) failed to mention the effects of LAAM, or lack thereof, on the cardiovascular system.

In summary, LAAM and its major metabolites nor-LAAM and dinor-LAAM have significant effects on the heart. Inotropic and chronotropic responses to LAAM have been observed in the isolated guinea pig atrium preparation at concentrations similar to the plasma concentrations observed in patients chronically receiving LAAM. In vivo cardiovascular actions by LAAM have been reported in experimental animals and scattered reports of such actions exist in the clinical literature. Taken collectively, the findings indicate that a careful examination of the in vivo cardiovascular actions of LAAM and its metabolites and their mechanisms is warranted.

The research in this dissertation shows that LAAM produces significant cardiovascular effects in vivo which may, under certain circumstances, alter homeostasis. Before outlining the specific aims of the project, I will review the literature which deals with known cardiovascular effects of other narcotic agents.

C. Cardiovascular Effects of Narcotic Analgesics

Morphine is considered the prototype narcotic agent because of qualitative similarities between its analgesic actions and those of other narcotic agents. As a result of this, the cardiovascular properties of morphine are often considered to apply to all narcotic analgesics. Two general cardiovascular effects of morphine are recognized; bradycardia and hypotension.

Early studies of the cardiac effects of morphine focused on heart rate more than cardiac mechanical function. These reports showed that the responses to morphine were as diverse as the various animal species and doses of morphine tested. In the frog, Junkmann (1925) observed

effects ranging from no change to a marked slowing of the heart after 10-30 mg/kg morphine s.c. Other investigators reported slight increases in heart rate followed by decreases (Hale, 1909). In the isolated perfused frog heart, morphine reportedly increased heart rate at a concentration of $1x10^{-4}$M and decreased it at higher concentrations (Hale, 1909). Morphine, 2 mg/kg s.c., usually decreased heart rate in rabbits (Eddy, 1932). In the Langendorff preparation, morphine has been reported to either increase and then decrease ($1x10^{-4}$M) or only decrease ($1x10^{-3}$M) the rate of beating of the rabbit heart (Vinci, 1907; Pennetti, 1926). The heart of the dog was usually slowed by morphine (4 mg/kg i.v.) (Van Egmond, 1911). In the cat, acceleration of the heart was reported to be the predominant response to morphine (Eddy, 1932). However, morphine caused stimulation followed by depression at low concentrations ($1x10^{-4}$M) and depression only at higher concentrations in the isolated cat heart (Vinci, 1907). In man, a general pattern of acceleration followed by depression has been reported (Eddy, 1941). However, the magnitudes of these effects in man varied greatly and did not appear to be dependent on the dose of morphine given (Anderson, 1929; Resnik et al., 1935; Eddy, 1941).

Investigations were made into the mechanisms for morphine induced bradycardia. In all species examined, this effect was reversed by atropine (Hale, 1909; Van Egmond, 1911; Eddy, 1932; Resnik et al., 1935). Increased vagal activity was suggested as the mechanism of action since cardiac effects are observed in vitro only at concentrations much higher than those attained during morphine use in vivo (see above). Robbins and coworkers (1939) undertook a detailed examination

of the site of action whereby morphine increased vagal activity in the dog. They concluded that morphine acted primarily on the medulla to cause its effect on heart rate. In all cases the bradycardia was eliminated by vagal section. However, they could not determine what interactions might occur between the respiratory effects of morphine observed in conscious animals and the effects on the medulla which they observed. Anesthetized animals were used in their experiments. Later studies have confirmed that narcotics such as morphine (5-10 mg/kg), fentanyl (5-50 μg/kg), and dextromoramide (10-200 μg/kg) can slow the heart through a medullary action to increase vagal activity (Kayaalp and Kaymakcalan, 1966; Fennessey and Rattray, 1969; Laubie et al., 1974).

In the only early experiment examining the effect of morphine on mechanical function, Hale (1909) reported that morphine ($1x10^{-3}$M) decreased contractile height in the frog Langendorff preparation. Other studies considered effects of morphine on mechanical function indirectly by examining the effect of morphine on cardiac output (Hamilton et al., 1932; Resnik et al., 1935) . It was concluded that neurally induced changes in heart rate, not changes in mechanical function, were responsible for the minor changes in cardiac output observed. The consensus was that narcotics, as a drug class, had little if any, direct action on the heart.

In the late 1950's, many new synthetic narcotics became available (Jaffe and Martin, 1975). The potential usefulness of these agents as analgesics in cardiac patients led to the study of their cardiac actions. Sugioka and coworkers (1957) reported that meperidine caused long-lasting decreases in contractile force in conscious dogs. They

concluded that meperidine acted directly on the heart since this effect was observed in dogs where neural influences on the heart had been interupted. More recently, Strauer (1972) compared the effects of morphine, piritramide, meperidine, and fentanyl on the mechanical function of isolated cat papillary muscle preparations. At concentrations ranging from 0.03 mM to 3.0 mM, all four drugs produced decreases in isotonic muscle shortening (Δl), rate of isotonic shortening (dl/dt_{max}), and rate of tension development (dT/dt_{max}). For morphine, piritramide, and fentanyl, their cardiodepressant effects at equianalgesic concentrations were similar. However, although meperidine is 1/5 to 1/10 as potent as morphine as an analgesic, it was 100-200 times more potent with regard to its cardiodepressant properties. In a later study, the cardiac effects of pentazocine, morphine and meperidine were compared (Strauer, 1974). Pentazocine, 1/3 as potent an analgesic agent as morphine, was 50 times more potent as a cardiodepressant than an equianalgesic dose of morphine. In summary, not all narcotics are similar to morphine with regard to their cardiac actions and, in fact, equianalgesic doses of certain narcotics may be more potent cardiodepressants than morphine.

Direct cardiac actions have been demonstrated for the structural analogue of methadone, propoxyphene, and its major metabolite nor-propoxyphene. Propoxyphene and nor-propoxyphene produced elongation of the P-R and QRS segments of the electrocardiogram in conscious rabbits (Lund-Jacobsen, 1978). Plasma, but not CNS, levels of the drugs correlated with the P-R and QRS segment duration changes. Distribution studies have shown that propoxyphene and nor-propoxyphene reach higher concentrations in heart than in brain or plasma after p.o., s.c., or

i.v. administration (Emmerson et al., 1967). The data suggest that these drugs act by a direct effect on the heart. Holland and Steinberg (1979) have studied the effects of propoxyphene and nor-propoxyphene on canine cardiac conducting tissue both in vitro and in vivo. Both agents caused significant depression of cardiac conduction in myocardial tissue. In some cases drug administration led to second degree heart block. The authors suggest that although these effects most likely do not occur at normal therapeutic doses, they may be important in acute overdose situations.

In addition to effects on the heart, morphine i.v. causes an immediate and short-lasting decrease in blood pressure, followed by a more prolonged (1-4 hr) hypotension. Morphine has been reported to produce hypotension in mice (Bonsmann, 1931), rabbits, rats, guinea pigs, cats, and dogs (Schmidt and Livingston, 1933). Most early studies concerning morphine induced hypotension were done in dogs and cats since independence of the hypotensive and respiratory depressant effects of morphine had been demonstrated in these species (Bogert et al., 1916).

In vivo, morphine was reported to cause dilation of cerebral (Schmidt, 1934), coronary (Gruber and Robinson, 1929) and cutaneous (Schmidt and Livingston, 1933) vascular beds in early experiments. More recent investigations have confirmed these findings (Henney et al., 1966; Zelis et al., 1974; Leaman et al., 1978) with the exception of the coronary bed, where constriction also has been reported (Vatner et al., 1975). Studies of the effects of morphine on excised vessels are inconclusive (Macht, 1914, 1915; Fujimori, 1933; Grundy, 1968; Flaim et al., 1977). Since morphine has minimal effects on cardiac

output (Hamilton et al., 1932; Resnik et al., 1935), the data above suggest that vasodilation is responsible for morphine induced hypotension.

In the early 1930's, several unsuccessful attempts were made to obtain evidence that the vasodilation caused by morphine was due to central vasomotor center depression (Schmidt and Livingston, 1933; Raab and Friedman, 1936). Reasons for these failures may have been the lack of understanding of central cardiovascular control at that time and insufficient control for respiratory effects which complicate data interpretation. As a consequence, prevailing opinion was that morphine was not a central vasomotor depressant. By the mid-1950's, histamine release was the established mechanism for the short-lasting hypotension following morphine i.v. (Evans et al., 1952). This mechanism could not explain the prolonged hypotensive effect, however, and a central mechanism was again suggested. Lack of evidence for a central action by narcotics on the vasomotor center persisted until about 1970 when several reports appeared reviving the hypothesis (Eckenhoff and Oech, 1960; Mansour et al., 1970; Lowenstein et al., 1972). Several narcotics including morphine (5-10 mg/kg), fentanyl (5-50 μg/kg), dextromoramide (10-200 μg/kg) and the morphinomimetic peptides β-endorphin and [d-ala^2]-met-enkephalin (50-500 μg/kg), have since been shown to decrease blood pressure by a central action to decrease sympathetic outflow (Laubie et al., 1974, 1977a,b; Daskalopoulos et al., 1975; Gomes et al., 1976).

In summary, morphine and narcotics in general have been shown to produce a hypotension, lasting several hours, which can be attributed, in part, to a depression of the vasomotor center. Bradycardia observed after narcotic administration is, in part, the result of a central action to increase vagal tone. In addition to effects involving the autonomic nervous system, several narcotics including methadone, propoxyphene and meperidine may exert direct actions on cardiac function. These compounds are structurally different from morphine and appear to have different cardiovascular actions. Methadone, propoxyphene and norpropoxyphene produce both inotropic and chronotropic responses in isolated cardiac tissue at concentrations at which morphine has no significant effect. LAAM and its metabolites are structural analogues of methadone. Since LAAM also produces cardiac effects, the data suggest that there may be a structure-activity relationship between narcotics and their cardiac effects. The ability of some, but not all, narcotics to produce cardiac effects suggests a heterogeneity of cardiovascular actions within the narcotic drug class. Another observation is that analgesic potency does not always correlate with cardiodepressant potency.

D. Specific Objectives

In vitro studies have shown that LAAM, nor-LAAM, and dinor-LAAM are capable of producing significant inotropic and chronotropic effects. The objective of the present work was to examine, and identify mechanisms for, the *in vivo* cardiovascular actions of LAAM and its metabolites.

The similarities and differences between LAAM and its metabolites with regard to their cardiovascular effects were examined first. Blood pressure, heart rate, contractile force, and Lead II electrocardiogram, as well as the response to bilateral carotid artery occlusion were measured. Additional experiments were performed to determine the relative contribution of cardiac and vascular changes to the effects of LAAM on arterial blood pressure.

The second set of experiments examined the contribution of the effects of LAAM on the sympathetic and parasympathetic nervous systems to the cardiovascular actions of LAAM. The contribution of direct effects of LAAM on the heart and vasculature to the cardiovascular effects of LAAM also was examined. An additional objective of these experiments was to more closely examine the mechanism for the chronotropic effects of LAAM. The negative chronotropic action of LAAM is antagonized both *in vitro* and *in vivo* by atropine. Therefore, it was determined whether LAAM or its metabolites had any significant anticholinesterase activity and, if so, whether this anticholinesterase effect could have contributed to the negative chronotropic activity of LAAM.

The third set of experiments was designed to determine the effects of LAAM on the peripheral sympathetic nervous system. In addition, the mechanisms for the observed effects and the possible contribution of these effects to the cardiovascular actions of LAAM were examined.

The cardiovascular actions of LAAM in the absence and presence of the narcotic antagonist naltrexone were examined in the final set of experiments.

MATERIALS AND METHODS

A. Materials

The drugs l-alpha-acetylmethadol (LAAM), l-alpha-acetylnormethadol (nor-LAAM), l-alpha-acetyldinormethadol (dinor-LAAM) and naltrexone, all in the form of their hydrochloride salts, were gifts from the National Institute for Drug Abuse, Research Triangle Park, North Carolina.

[^{14}C-Acetyl]-choline iodide was purchased from New England Nuclear Co., Boston, Massachusetts.

l-Epinephrine bitartrate (adrenaline), 6-hydroxydopamine hydrobromide, heparin sodium salt Grade I, methacholine chloride, acetylcholine chloride, butyrylcholinesterase from horse serum, acetylcholinesterase from bovine erythrocytes, carbamyl β-methylcholine chloride (bethanechol), eserine sulfate (physostigmine), albumin (bovine fraction V powder), tetraphenyl boron sodium, tyramine hydrochloride and atropine sulfate were purchased from Sigma Chemical Co., St. Louis, Missouri. 6-Hydroxydopamine hydrobromide also was purchased from Aldrich Chemical Co., Milwaukee, Wisconsin.

Practolol was a gift from Ayerst Laboratories Inc., New York, New York.

Cardio-green (indocyanine green) was purchased from Hynson, Westcott, and Dunning, Baltimore, Maryland. All other chemicals used were of the standard analytical grade.

B. Experiments to Compare the Cardiovascular Effects of LAAM, nor-LAAM, and dinor-LAAM

1. Cumulative Dose-Response Experiments in Dogs

Mongrel dogs of either sex, 8.9-12.2 kg, were used in these experiments. The animals were fasted for 24 hours before the experiment in order to decrease the excessive loose defecation which occurs following LAAM administration (Stickney and Schwartz, unreported observations). The animals were anesthetized with sodium pentobarbital (30 mg/kg) i.v. and artificially respired with room air via a tracheal cannula. Positive end-expiratory pressure (PEEP) was not used in any of the experiments in this dissertation. However, blood gases remained stable throughout the experiment in all cases where blood gases were monitored. A femoral artery was cannulated and arterial blood pressure (BP) was monitored via a Statham pressure transducer (Model 230 Db). The Lead II electrocardiogram (ECG) also was monitored. A right thoracotomy was performed at the level of the fourth intercostal space and a pericardial sling prepared. Right ventricular contractile force (CF) was then measured via a Walton-Brodie strain gauge sutured to the right ventricle. Heart rate (HR) was monitored using a tachograph (Grass Model 7P4F) triggered by the contractile force signal. All four parameters, BP, HR, CF and ECG were continuously recorded on a Grass polygraph (Model 7) (Quincy, Mass.) throughout each experiment. In addition, the common carotid arteries were isolated at the cervical level to enable the performance of one minute bilateral carotid occlusions (BLCO) at intervals throughout each experiment.

A 30 min stabilization period followed the completion of surgery. At the end of this period, control values for BP, HR, and CF

were established and a control ECG trace was obtained. The control response to BLCO also was determined at this time.

LAAM, nor-LAAM and dinor-LAAM were given i.v. to groups of 5 animals (a total of 15 animals) in a cumulative dose fashion using the following protocol. Once control values for all the parameters being monitored were established, the appropriate drug was given in an amount sufficient to obtain the desired initial dose. BLCO was performed 15 min after administration of the drug. Following the BLCO, the animal was allowed to return to preocclusion status before the next drug injection was given. The amount of drug in the next and subsequent injections was calculated, taking into account the amount of drug already given, so the next desired total dose could be obtained. This procedure was repeated until the highest total dose desired was reached. All doses of LAAM, nor-LAAM and dinor-LAAM were equimolar, thereby allowing comparison of relative potencies among LAAM and congeners.

The doses of LAAM, nor-LAAM and dinor-LAAM used in these experiments were calculated in an attempt to approximate the plasma levels of LAAM or its metabolites as they are found during chronic LAAM administration in humans. Although there is not much information available concerning plasma drug levels during chronic LAAM administration, some studies using small numbers of patients have been published. The earliest report of plasma levels in patients chronically receiving LAAM described only the concentrations of nor-LAAM and dinor-LAAM in 3 patients previously stabilized on methadone (Billings et al., 1974). In these subjects receiving 100 mg LAAM orally 3 times/week, combined plasma concentrations of nor-LAAM and dinor-LAAM averaged approximately 1.3×10^{-6}M. It should be noted, however, that significantly higher

concentrations of the two metabolites were periodically observed. Kaiko and Inturrisi (1975) studied the relationship between the miotic effects of LAAM administration and plasma levels of LAAM, nor-LAAM and dinor-LAAM. In eight subjects receiving 50 mg LAAM 3 times/week for 4 to 25 weeks, combined plasma concentrations following a single oral dose approached 1.5×10^{-6}M. Henderson and co-workers (1977a) also examined the plasma concentrations of LAAM, nor-LAAM and dinor-LAAM in 10 male patients receiving 60-85 mg LAAM 3 times/week. Plasma concentrations for LAAM, nor-LAAM and dinor-LAAM were 6×10^{-7}M, 1×10^{-6}M, and 6×10^{-7}M, respectively. A subsequent study (Henderson et al., 1977b) involving 11 male subjects receiving .62 to 1 mg/kg LAAM 3 times/week showed combined plasma concentrations for LAAM, nor-LAAM, and dinor-LAAM 48 hours after the tenth dose to be approximately 1.2×10^{-6}M. Concentrations went as high as 5×10^{-6}M in some patients.

The above studies indicate that plasma concentrations for LAAM, nor-LAAM, and dinor-LAAM combined are in the neighborhood of 2×10^{-6}M. When these studies were performed, lower doses of LAAM than are currently employed in the clinic were used. Therefore, the plasma concentrations probably underestimate plasma concentrations observed today. Nonetheless, the published plasma levels which were available at the beginning of the study were used in determining the doses of LAAM which were used in this study. Although little pharmacokinetic data were available when the current studies were begun, evidence did exist suggesting that the volume of distribution of LAAM was equal to total body water (Misra and Mule, 1975). Therefore, the doses of LAAM, nor-LAAM, and dinor-LAAM used in the present studies were calculated, based on a distribution to total body water, so that the obtained plasma

concentration would cover a range around the plasma concentrations reported in the literature. In these experiments, doses were used which were calculated to produce plasma concentrations in the range from $1x10^{-9}$M to $2x10^{-5}$M.

The use of cumulative dose-response methods allowed the generation of a complete dose response curve in a single animal. Adequate precautions were taken to maintain the validity of this method. Pilot experiments using saline vehicle injections showed that none of the parameters being monitored significantly changed during the course of the experiment. Secondly, one might question whether metabolism of LAAM could result in conversion of significant amounts of LAAM to nor-LAAM and dinor-LAAM. Calculations based on a maximum time of 3 hours from the time of the first LAAM injection to the end of the experiment and the $T_{\frac{1}{2}}$ for LAAM indicate a maximum conversion of 24%. However, when it is considered that better than 90% of the total drug given is administered during the last hour of the experiment, it appears that significant metabolism of LAAM to its metabolites is not a concern.

In the graphical presentation of the data in this section of the results, all doses are expressed as μmoles/kg to facilitate drug-to-drug and pretreatment-to-pretreatment comparison. The term μmoles/kg/7.0 in figure legends is a concise way of indicating that each dose on the abscissa is to be multiplied by 7. In all discussions of the data in the text, drug doses will be expressed as μg or mg/kg. Table 1 shows the doses of LAAM, nor-LAAM and dinor-LAAM used in μmoles/kg and then in μg or mg/kg equivalents. The molecular weights of the compounds (as the HCl salts) are: LAAM, 391.5; nor-LAAM, 376.5; dinor-LAAM, 361.5.

TABLE 1

Dose Equivalents for LAAM, nor-LAAM, and dinor-LAAM

μmoles/kg	LAAM	nor-LAAM	dinor-LAAM
$7.0x10^{-4}$	0.27 μg/kg	0.26 μg/kg	0.25 μg/kg
$7.0x10^{-3}$	2.7 μg/kg	2.6 μg/kg	2.5 μg/kg
$7.0x10^{-2}$	27.3 μg/kg	26.4 μg/kg	25.3 μg/kg
$3.5x10^{-1}$	---	0.13 mg/kg	---
$7.0x10^{-1}$	0.27 mg/kg	0.26 mg/kg	0.25 mg/kg
3.5	1.4 mg/kg	1.3 mg/kg	1.3 mg/kg
7.0	2.7 mg/kg	2.6 mg/kg	2.5 mg/kg
$1.4x10^{1}$	5.5 mg/kg	5.3 mg/kg	5.1 mg/kg

2. LAAM Dose-Response Experiments in Cats

Cats, 2.0-4.5 kg, were used in these experiments. The animals were fasted for 24 hours before the experiment. Animals were anesthetized with pentobarbital sodium (35 mg/kg) i.p. and artificially respired via a tracheal cannula. A femoral artery was cannulated and arterial blood pressure (BP) was monitored via a Statham pressure transducer (Model 230Db). The Lead II electrocardiogram (ECG) was recorded. A midsternal thoracotomy was performed and a pericardial sling prepared. Right ventricular contractile force (CF) was monitored via a small Walton-Brodie stain-gauge arch sutured to the right ventricle. Heart rate (HR) was monitored using a tachograph (Grass Model 7P4F) triggered by the contractile force signal. All four parameters, BP, HR, CF, and ECG were continuously recorded on a Grass polygraph (Model 7) throughout each experiment.

A 30 minute stabilization period followed the completion of surgery. At the end of this period, control values for BP, HR, and CF were established and a control ECG trace was obtained. LAAM was then given in a cumulative dose fashion using the protocol outlined above.

3. Experiments to Determine the Relative Contribution of Cardiac and Vascular Changes to the Effects of LAAM on Blood Pressure

Mongrel dogs, 8.0-14.5 kg, were used in these experiments. The animals were fasted for 24 hours prior to the experiment. The animals were anesthetized with pentobarbital sodium (30 mg/kg) i.v. and artifically respired with room air via a tracheal cannula. A femoral artery was cannulated and arterial blood pressure (BP) was monitored via a Statham pressure transducer (Model 230Db). A femoral vein was

cannulated for drug administration. A second femoral artery was cannulated for withdrawal of blood during cardiac output determinations. The withdrawal system was heparinized to prevent clotting of the withdrawn blood. A cannula also was passed through the right jugular vein into the right atrium. This cannula would be used for dye delivery during cardiac output determinations. Lead II electrocardiogram (ECG) and heart rate (HR) also were monitored. HR was determined by a tachograph (Grass Model 7P4F) triggered by the ECG signal. BP, HR and ECG were continuously recorded by a polygraph (Grass Model 7). Total peripheral resistance (TPR) was calculated assuming negligible right atrial pressure using the formula (Milnor, 1974):

$$\text{TPR (dyne·sec/cm}^5\text{)} = \frac{\text{mean BP (mmHg)}}{\text{cardiac output (L/min)}} \times 80$$

A 30 minute stabilization period followed the completion of surgery.

At the end of this period, BP, HR and a control ECG trace were recorded. Duplicate control determinations of cardiac output were made until two consecutive determinations agreed within 10%. Cardiac output was measured using the dye-indicator-dilution method with a Lexington Instrument Densitometer and Cardiac Output Computer (Lexington, MA). Cardiac output was determined by injection into the right atrium of 2.5 mg of indocyanine green dissolved in 1 ml of solvent. This was flushed in with 10 ml 0.9% saline. Simultaneously, arterial blood was withdrawn and passed through a cuvette in the densitometer head at the rate of 15 ml/min. The computer analyzed the dye curve and, if it was exponential, gave a digital readout of cardiac output in liters per min. Withdrawn blood was then immediately reinfused. Following the control cardiac output determinations, 1.4 mg/kg LAAM was given over 2 minutes. Twenty

minutes after the LAAM injection was begun, cardiac output was determined and BP, HR and an ECG trace were recorded. Thirty minutes following the first injection, another 1.4 mg/kg LAAM was given, making a total dose of 2.7 mg/kg. Twenty minutes following the injection, cardiac output was determined and BP, HR and and an ECG trace were recorded. Thirty minutes following the second injection, another 2.7 mg/kg LAAM was given, making a total dose of 5.5 mg/kg. Again 20 minutes after the injection, cardiac output was determined and BP, HR and an ECG trace were recorded. Control experiments where only LAAM vehicle (0.9% saline) was given showed no significant change in BP, HR, ECG or cardiac output over the experimental time period.

In another set of experiments, the effects of cumulative doses of practolol on BP, HR, TPR and cardiac output were examined. The total doses of practolol examined were 1.0, 2.5, and 5.0 mg/kg. These doses were given in the same manner as described for LAAM above. Since practolol is a cardioselective β-adrenergic blocking agent, any effects on BP observed after practolol would be attributable to changes in cardiac output and not vascular actions of the drug. By comparison of the effects of LAAM and practolol on BP against their actions on cardiac output, the possible vascular effects of LAAM contributing to effects on BP could be indirectly examined.

C. Experiments to Determine the Contribution of Effects of LAAM on the Autonomic Nervous System to LAAM's Cardiovascular Actions

This set of experiments is divided into two categories, *in vivo* and *in vitro* experiments.

1. In Vivo Experiments

The contribution of the effects of LAAM on the sympathetic and parasympathetic nervous system to the cardiovascular actions of LAAM was investigated. Possible direct actions of LAAM on the heart and vasculature also were examined. LAAM was given i.v. in the cumulative dose fashion described above to mongrel dogs, 8.9-12.2 kg. Groups of 5 animals were vagotomized, sympathectomized, or both vagotomized and sympathectomized by the methods described below prior to LAAM administration.

a. Vagotomized Animals

Five dogs were anesthetized and surgically prepared for monitoring BP, HR, CF and ECG as described earlier. In addition, the animals were vagotomized by bilateral sectioning of the vagi at the cervical level. Vagotomy did not significantly change the heart rate in this group of animals. The heart rate (beats/min ± SEM) before vagotomy was 142.8±7.4 and 143.2±9.7 after vagotomy. Note that the heart rate before vagotomy is significantly less than the control heart rates in "intact" animals (Figure 7). Additional vagotomy experiments have been performed in animals with higher HR and the results were the same as those shown in Figure 7. Cumulative dose-response curves for LAAM then were generated as described above. For purposes of data analysis, post-vagotomy BP, HR, and CF values prior to LAAM administration were considered as control values for these parameters.

b. Sympathectomized Animals

Five animals were chemically sympathectomized (SYMX) by administering a total dose of 50 mg/kg of 6-hydroxydopamine, i.v., over a 6 day period (Stickney, 1976). Control animals received vehicle only in the same schedule. On the 7th day the animals were anesthetized and surgically prepared for monitoring BP, HR, CF, and ECG as described earlier. Cumulative dose-response curves then were obtained in these animals as described above.

SYMX was verified biochemically by analysis of heart and spleen catecholamine concentrations, physiologically by observing the responses to BLCO, and pharmacologically by examination of the pressor response to 5 μg/kg tyramine, i.v. Tissue norepinephrine concentrations were determined using the method of Anton and Sayre (1962). Data verifying successful sympathectomy are shown in Table 2.

c. Sympathectomized and Vagotomized Animals

In five animals the vagi were bilaterally sectioned following chemical sympathectomy (both procedures described above). Dose-response curves for LAAM then were generated as described above. In these experiments post-vagotomy BP, HR, and CF values prior to LAAM administration were considered as control values for these parameters.

2. In Vitro Experiments

The effect of LAAM and its metabolites on cholinesterase activity was examined in vitro. In addition, the effect of LAAM on the chronotropic response to bethanechol in the isolated guinea pig atrium preparation was examined.

TABLE 2

Verification of Chemical Sympathectomy

	Control	6-OH Dopamine
Pressor Response[a] to BLCO[b]	41.0±9.8/36.6±7.4	7.2±1.4/7.4±1.9
Pressor Response[a] to Tyramine	70.0±4.2/60.2±4.7	7.0±1.2/6.0±1.5
Norepinephrine Content[c]		
Heart	1.75±0.13	0.11±0.04
Spleen	3.87±0.24	0.15±0.06

[a] $\bar{X}$ ±SEM; mmHg systolic/mmHg diastolic. For resting mean blood pressure refer to Figure 6.

[b] Bilateral Carotid Artery Occlusion.

[c] $\bar{X}$ ± SEM; µg/g tissue, not corrected for assay recovery.

a. In Vitro Enzyme Studies

Cholinesterase activity in the present study was determined by monitoring the rate of enzymatic production of ^{14}C-acetate from the hydrolysis of [^{14}C-acetyl]choline iodide according to the method of Fonnum (1969). Briefly, a total incubation volume of 50 µl contained as final concentration: 3 mM acetylcholine (ACh) iodide which included 200,000 DPM [^{14}C-acetyl]choline iodide; 20 mM sodium phosphate buffer, pH 7.2; 10 mM $MgCl_2$; the enzyme being studied; and the drug being studied at the appropriate concentration. The reaction was monitored over a 30 min period at 37°C during which time acetate production was linear with time in all experiments. In each experiment there were six determinations of acetate production in individual tubes at each of the following time points: 2, 5, 10, 15, 20, and 30 min. These six determinations were subdivided as follows: 2-control tubes with no drug added, 2-blanks tubes containing $1x10^{-4}$M physostigmine in the reaction mixture, 2-treated tubes containing the drug being studied at the appropriate concentration. The average blank value at each time point was subtracted from the appropriate control and treated values to determine the amount of ^{14}C-acetate enzymatically produced. The rate of hydrolysis for control and treated data was then determined by least square analysis of nmoles ACh hydrolyzed/min. In each experiment the amount of enzyme in the incubation mixture was adjusted so that 5-10% of the total substrate present would be hydrolyzed over the 30 minute incubation period after the activity had been determined in preliminary experiments.

The effects of several drugs on cholinesterase activity from four different enzyme sources were studied: 1) purified acetylcholine hydrolyase (E.C. 3.1.1.7) from bovine erythrocytes, 2) purified butyrylcholine esterase (E.C. 3.1.1.8) from horse serum, 3) heparinized guinea pig plasma, and 4) guinea pig heart homogenate.

Purified enzymes were prepared in a solution of sodium phosphate buffer, pH 7.2, containing bovine serum albumin (0.8 mg/ml) and $MgCl_2$ at a concentration necessary to insure the final concentrations outlined above.

Plasma samples were obtained from guinea pigs by collecting blood in heparinized centrifuge tubes on ice following decapitation. The blood was centrifuged at 3,000 RPM at 4°C for 20 minutes. The plasma was then dialyzed overnight against 200 volumes of the sodium phosphate buffer, pH 7.2, containing $MgCl_2$. At the time of use appropriate dilutions of the plasma were made with the buffer.

Guinea pigs heart homogenates were made by decapitating the guinea pigs and quickly removing the hearts. The interatrial and interventricular septa, as well as the left and right atria were dissected from the hearts and homogenized in 9 volumes of the sodium phosphate buffer, pH 7.2, containing $MgCl_2$ and 0.5% triton X-100. The homogenate was dialyzed against 200 volumes of the buffer minus the triton X-100 overnight at 4°C before use.

Data from purified enzyme and heart homogenate experiments were expressed in final form as nmoles ACh hydrolyzed/min/mg protein. Protein content was determined by the method of Lowry *et al.* (1951). Plasma data were expressed as nmoles ACh hydrolyzed/min/ml

plasma. All data are presented as percent of control to facilitate comparison of experimental results in different enzyme systems.

b. Isolated Atria Studies

A detailed presentation of the methods used in these experiments has been published elsewhere (Stickney, 1977a). Only a brief summary will be given here. All experiments were carried out on right atria of guinea pigs, 350-550 gms, of either sex. Animals were stunned by a blow to the head and the thoracic cage quickly opened. The heart was removed and placed in pre-oxygenated modified Krebs-Henseleit solution where it was rinsed and the atria cut away from the ventricles. The atria were transferred to a fresh physiological solution that was continuously oxygenated. They were carefully trimmed and then cut apart. The right atrium was placed in an atria holder, lowered into a 90 ml bath of modified Krebs-Henseleit solution, connected to a Grass FT-03C transducer, and allowed to equilibrate for 1 hour. The bath was continuously bubbled with 95% O_2/5% CO_2 and maintained at pH 7.4 and a temperature of 30°C. Contractile frequency was used to monitor the rate of atrial beating.

The effects of LAAM and physostigmine on the chronotropic action of bethanechol were studied in the isolated right atrium preparation. All concentration-effect curves were obtained in a cumulative manner (Stickney, 1977a). A stock solution was prepared for bethanechol and appropriate dilutions were made so that the drug could be added to the bath to produce increasing molar concentrations. The following concentrations were studied: $1x10^{-9}$, $1x10^{-8}$, $5x10^{-8}$, $1x10^{-7}$, $5x10^{-7}$, $1x10^{-6}$, $5x10^{-6}$, $1x10^{-5}$ and $5x10^{-5}$. The appropriate concentration of

LAAM or physostigmine was added to the bath 15 minutes before a bethanechol concentration-effect curve was generated in the interaction experiments. Glass distilled water was added in control experiments. Only one concentration-effect curve could be obtained with a given atrium.

D. Experiments to Determine the Effects of LAAM on the Peripheral Sympathetic Nervous System: Effects of LAAM on the Cat Nictitating Membrane Preparation

Cats, 2.0-4.5 kg, were used in these experiments. The animals were fasted for 24 hours prior to the experiment. Animals were anesthetized with pentobarbital sodium (35 mg/kg) i.p. and artificially respired with room air. A femoral artery was cannulated and arterial blood pressure monitored via a Statham pressure transducer (Model 230Db). The Lead II electrocardiogram also was monitored. The animal's head was mounted in a David Kopf Stereotaxic Apparatus (Tujunga, CA). The animal was placed ventral side up and the neck opened. The trachea and esophagus were inverted through the mouth, giving access to the cervical sympathetic trunk, the superior cervical ganglion, and the postganglionic cervical sympathetic nerve. On the right side the preganglionic cervical sympathetic nerve was placed over bipolar platinum stimulating electrodes. The nerve was sectioned in the direction of the central nervous system. On the left side, the postganglionic superior cervical nerve was placed over bipolar stimulating electrodes. The postganglionic nerve was sectioned at its exit from the ganglion. A pool containing mineral oil was then formed in the neck and both nerves were submerged. Each nictitating membrane was attached with 3-0 silk suture to a force transducer (Grass Model FT03) with an initial tension of 7.5 gms. BP, ECG, and the nictitating membrane tensions were continuously recorded

on a polygraph (Grass Model 7). Following the completion of the preparatory procedures, a 30 minute stabilization period was allowed.

At the end of this period the experiment was begun. Control BP, heart rate (HR), and control frequency-response curves for the nictitating membranes to preganglionic and postganglionic nerve stimulation at 10 V, 0.5 msec duration, and frequencies of 0.5, 1.0, 3.0, 5.0, 10.0, 15.0 and 20.0 Hz were generated. Stimuli were delivered by a Grass stimulator (Model S88). In addition, the responses of the nictitating membranes to 5 μg/kg epinephrine, i.v., were monitored. Responses were elicited under the following experimental conditions:

1. Before and after cumulative doses of LAAM, i.v. from 27 μg/kg to 5.5 mg/kg in control and atropine (1.5 mg/kg i.v.) pretreated animals. Cumulative dose-response curves were generated in a manner similar to that described in section B. The initial dose of LAAM, 27 μg/kg, was given and 20 minutes was allowed for the animal to stabilize. At the end of this period BP, HR, and an ECG trace were recorded. Frequency-response curves of the nictitating membranes to nerve stimulation and the responses of the nictitating membranes to i.v. epinephrine also were determined at this time. Thirty minutes were allowed between successive dose of LAAM and the procedure described above was carried out after each LAAM dose. In atropine pretreated animals, responses to methacholine, 5 μg/kg i.v., were obtained before and after atropine. Only those experiments where the response to methacholine was completely blocked by atropine were used in analysis of the data.

2. The responses to both nerve stimulation and epinephrine were determined in animals before and after a bolus injection of either LAAM,

2.7 mg/kg, or morphine, 13 mg/kg. These experiments were repeated in animals which had been pretreated with naltrexone, 3.0 mg/kg s.c., 35 minutes prior to the narcotic injection. Subsequently these experiments were performed using LAAM doses of 5.5 and 8.2 mg/kg in an attempt to overcome the naltrexone antagonism.

E. Experiments to Determine the Involvement of Opiate Binding Sites in the Mechanisms for the Cardiovascular Effects of LAAM and nor-LAAM

Mongrel dogs, 8.0-15.5 kg, were used in these experiments. The animals were fasted for 24 hours prior to the experiment. Animals were anesthetized and surgically prepared in the same manner as in section B. BP, HR, CF, and ECG were monitored. Following a 30 minute stabilization period, control values for the parameters being observed were recorded. After this, one of three protocols was followed:

1. A bolus injection of LAAM, 1.4, 2.7, or 5.5 mg/kg or nor-LAAM, 0.27 mg/kg (n=3 each) was given over 10 minutes. BP, HR, CF, and ECG were recorded 20 minutes following the beginning of the drug injection and again 50 minutes following the injection. This time course allowed the maximum response to the drug to be observed.
2. Naltrexone was given, s.c., in a dose of 300 μg/kg. Thirty-five minutes following naltrexone administration, LAAM, 2.7 mg/kg or nor-LAAM, 0.27 mg/kg (n=3 each) was given as a bolus injection over 10 minutes. BP, HR, CF, and ECG were then recorded 20 minutes following the beginning of the drug injection and 50 minutes following the beginning of the injection. BP, HR, CF, and ECG recorded after naltrexone and before LAAM

or nor-LAAM were considered as control values in these experiments.

3. Naltrexone was given, s.c., in a dose of 5 μg/kg. Thirty-five minutes following naltrexone administration, LAAM was given over 10 minutes in a dose of 1.4, 2.7, or 5.5 mg/kg (n=3 each). BP, HR, CF, and ECG were then recorded 20 minutes following the beginning of the LAAM injection and 50 minutes following the beginning of the LAAM injection. BP, HR, CF, and ECG recorded after naltrexone and before LAAM were considered as control values in these experiments.

The dose of 5 μg/kg of naltrexone was not randomly chosen, but rather, was determined by preliminary experiments examining different doses of naltrexone. Table 3 shows the data from these experiments. At doses of naltrexone from 3 mg/kg to 10 μg/kg, essentially complete blockade was observed. The blood pressure response to LAAM was completely eliminated in this range. The heart rate data indicate that part of the negative chronotropic response cannot be blocked by naltrexone and, therefore, is not the result of LAAM interacting with opiate binding sites. Five μg/kg naltrexone, partially antagonized the effect of 2.7 mg/kg LAAM. Since one of the objectives of the antagonism experiments was to examine the nature of the antagonism, this dose of naltrexone was then used with both higher and lower doses of LAAM.

TABLE 3

Effects of Several Doses of Naltrexone on the Cardiovascular Responses to a Bolus Injection of LAAM (2.7 mg/kg) in the Dog

Naltrexone Pretreatment	Mean Blood Pressure[a] After LAAM	Heart Rate[a] After LAAM	Contractile Force[a] After LAAM
None	80.3±8.1[b,c]	50.6± 2.7[b,c]	67.0±4.8[b,c]
3.0 mg/kg	103.3	88.7	92.5
300 µg/kg	97.8±5.1[b]	87.8± 1.8[b,c]	86.5±11.5[b]
30 µg/kg	101.9	87.1	88.2
15 µg/kg	91.4	87.5	92.9
10 µg/kg	100.9	88.6	83.3
5 µg/kg	87.9±1.4[b,c]	75.4±11.4[b,c]	87.2±18.2[b]

[a] % of control. Combined control values for all animals ($\bar{X}$ ± SEM): mean blood pressure (mmHg), 131.1±3.8; heart rate (beats/min), 158.1±4.5, contractile force (gms), 68.8±3.9.

[b] $\bar{X}$ ± S.E.M., N=3.

[c] Significantly less than pre-LAAM control, $p<.05$.

F. Statistical Analyses

All data were analyzed using analysis of variance (Sokal and Rohlf, 1969). Difference between means were determined using either Tukey's test or Student-Neuman-Keuls test. In all cases, $p<.05$ was the level of significance chosen.

RESULTS

A. The Cardiovascular Effects of LAAM, nor-LAAM, and dinor-LAAM

Significant amounts of the metabolites nor-LAAM and dinor-LAAM are present in the plasma of patients chronically receiving LAAM (Kaiko and Inturrisi, 1975; Henderson et al., 1977a). For this reason, the cardiovascular effects of nor-LAAM and dinor-LAAM, as well as those of LAAM were examined. Intravenous administration of LAAM, or either of its major metabolites, consistently produced decreases in mean arterial blood pressure (BP), heart rate (HR) and contractile force (CF) in anesthetized dogs. The effect was characterized by an immediate, short-lived decrease in BP, HR and CF followed by a gradual, long-lasting decrease in these parameters occurring over 10-15 minutes. Qualitative and quantitative alterations in the Lead II electrocardiogram (ECG) also were observed. Responses following the intravenous administration of LAAM in a representative dog are shown in Figure 1. In this animal, BP HR, and CF were decreased to approximately 70, 55, and 70% of control respectively after 5.5 mg/kg LAAM, the highest dose given. In addition, the most commonly observed ECG alterations, increases in T wave amplitude and prolongation of the P-R interval, are both evident in Figure 1.

Cumulative dose-response curves for the effects of LAAM, nor-LAAM and dinor-LAAM on BP are shown in Figure 2. Although all 3 drugs

Figure 1. Cardiovascular responses to l-α-acetylmethadol in anesthetized dogs. Lead II electrocardiogram (ECG), heart rate, contractile force, and blood pressure were monitored continuously in this representative animal. The numbers along the bottom of the figure indicate the total dose (μmoles/kg) present in the animal at the time the records shown were obtained.

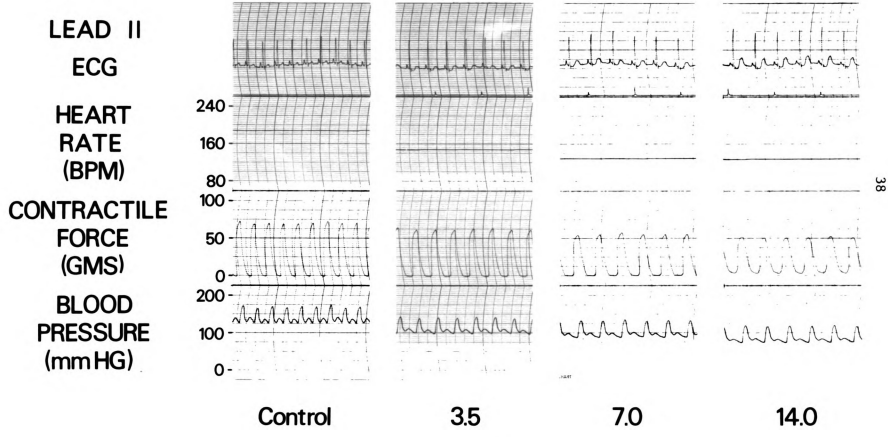
LEAD II
ECG
HEART
RATE
(BPM)
240
160
80
CONTRACTILE
FORCE
(GMS)
100
50
0
BLOOD
PRESSURE
(mm HG)
200
100
0
Control
3.5
7.0
14.0

Figure 1

Figure 2. Cumulative dose-response curves for the effects of LAAM, nor-LAAM and dinor-LAAM on mean arterial blood pressure in anesthetized dogs. Each dose represents the total amount of drug given at the time the measurements were made. In the upper right hand corner is the key for the symbols used in the curves. The asterisks (*) denote the threshold doses for significant decreases from respective control values, $p<.05$. Each data point represents the mean of 5 replications; the vertical lines represent the S.E.M.

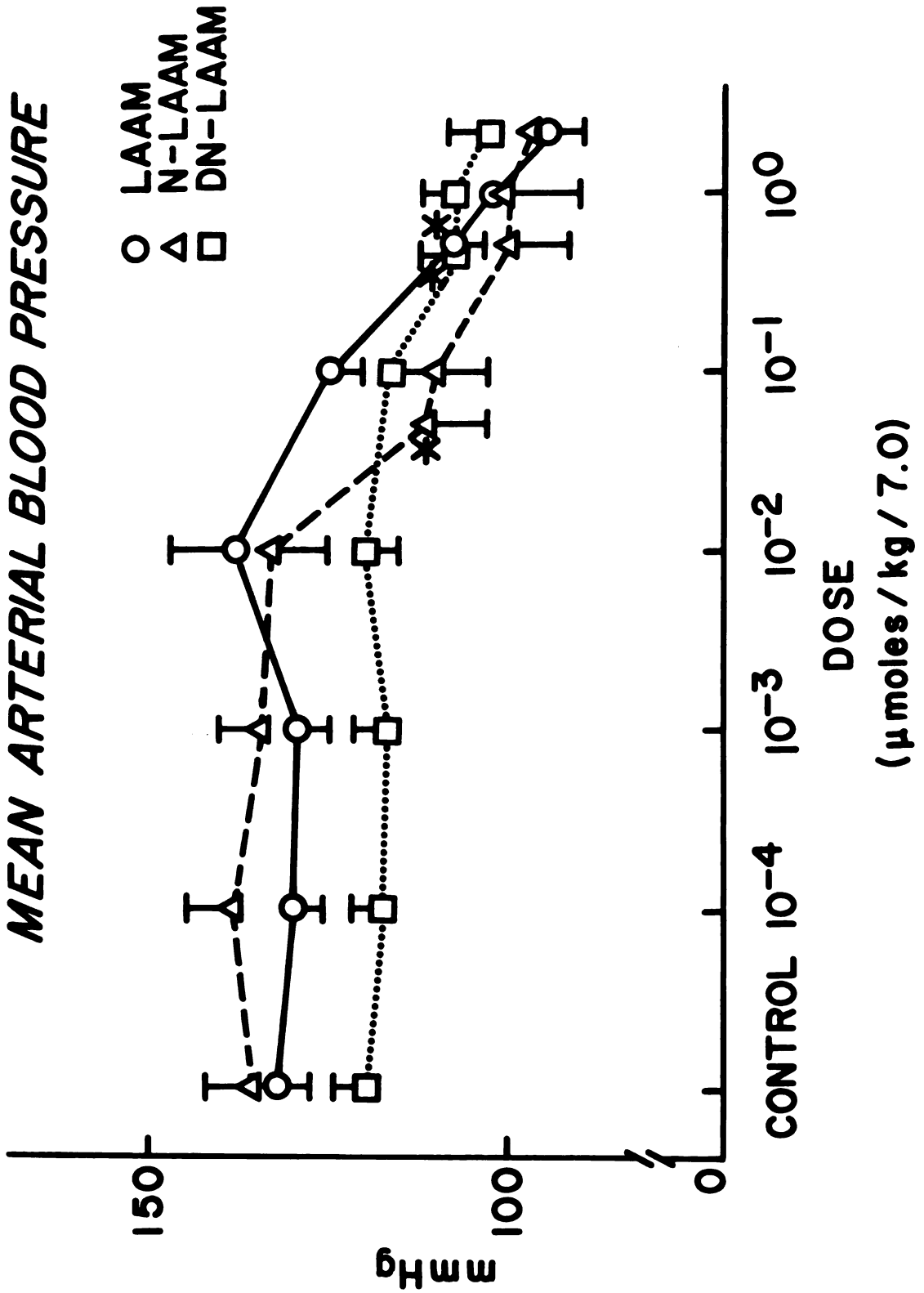
MEAN ARTERIAL BLOOD PRESSURE
LAAM
N-LAAM
DN-LAAM
150
100
0
mmHg
CONTROL
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^{0}
DOSE
(μmoles/kg/7.0)

Figure 2

significantly lower BP, some differences in potency were observed. LAAM and dinor-LAAM appeared similar in potency, the lowest doses which significantly decreased BP being 1.4 and 1.3 mg/kg, respectively. Nor-LAAM, on the other hand, produced significant decreases in BP after 0.13 mg/kg. The maximum doses of LAAM and nor-LAAM given had similar effects on BP, decreasing it to 71.4±4.2 and 61.0±7.5 percent of control ($\bar{X}$±S.E.M.), respectively. An equimolar dose of dinor-LAAM produced a smaller response, decreasing BP to 87.4±2.6 percent of control.

Significant decreases in HR were produced by LAAM and its major metabolites (Figure 3). Again, differences in potency were observed, with LAAM and dinor-LAAM producing significant decreases after 1.4 and 1.3 mg/kg, respectively, and nor-LAAM after 0.13 mg/kg. The highest dose of each drug produced approximately a 50% decrease in HR relative to the respective control value.

In addition to effects on BP and HR, LAAM, nor-LAAM and dinor-LAAM significantly decreased CF (Figure 4). The minimum doses required to produce significant decreases in contractile force were similar for all 3 drugs, 1.3 mg/kg. The highest dose of LAAM used produced a 32.5±7.4% decrease in CF whereas equimolar doses of nor-LAAM and dinor-LAAM produced 14.6±6.4 and 13.1±3.0% decreases, respectively.

In an attempt to assess the effects of LAAM and its metabolites on the reflex mechanisms which maintain cardiovascular homeostasis, the responses of BP, HR, and CF to bilateral carotid artery occlusion (BLCO) were examined before and after LAAM, nor-LAAM, and dinor-LAAM. The responses of BP, HR, and CF to BLCO were decreased by LAAM and its

Figure 3. Cumulative dose-response curves for the effects of LAAM, nor-LAAM and dinor-LAAM on heart rate in anesthetized dogs. Each dose represents the total amount of drug given at the time the measurements were made. In the upper right hand corner is the key for the symbols used in the curves. The asterisks (*) denote the threshold doses for significant decreases from respective control values, $p<.05$. Each data point represents the mean of 5 replications; the vertical lines represent the S.E.M.

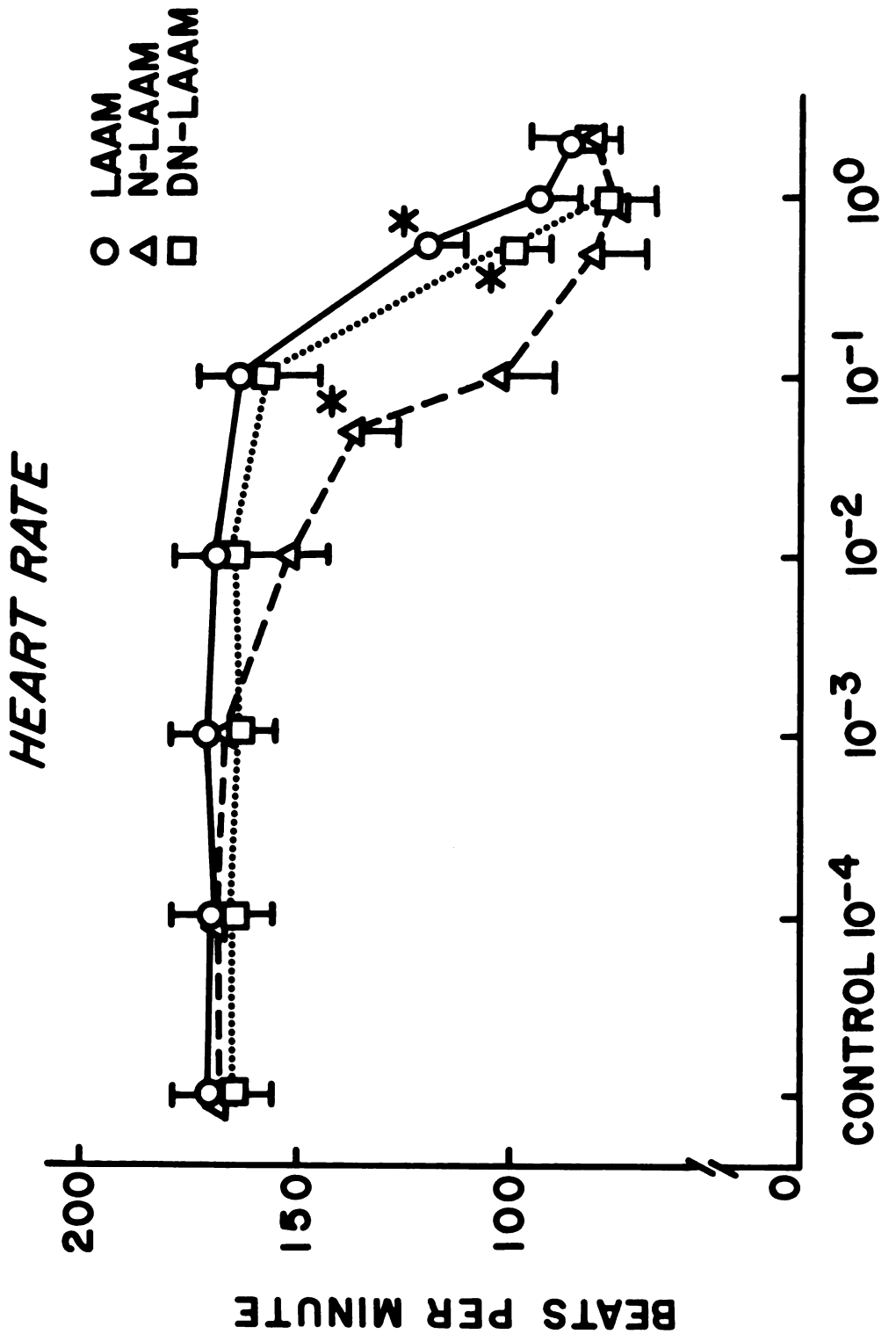
HEART RATE
LAAM
N-LAAM
DN-LAAM
BEATS PER MINUTE
200
150
100
0
CONTROL
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^{0}
DOSE
(μmoles/kg/7.0)

Figure 3

Figure 4. Cumulative dose-response curves for the effects of LAAM, nor-LAAM and dinor-LAAM on right ventricular contractile force in anesthetized dogs. Each dose represents the total amount of drug given at the time the measurements were made. In the upper right hand corner is the key for the symbols used in the curves. The asterisks (*) denote the threshold doses for significant decreases from respective control values, $p<.05$. Each data point represents the mean of 5 replications; the vertical lines represent the S.E.M.

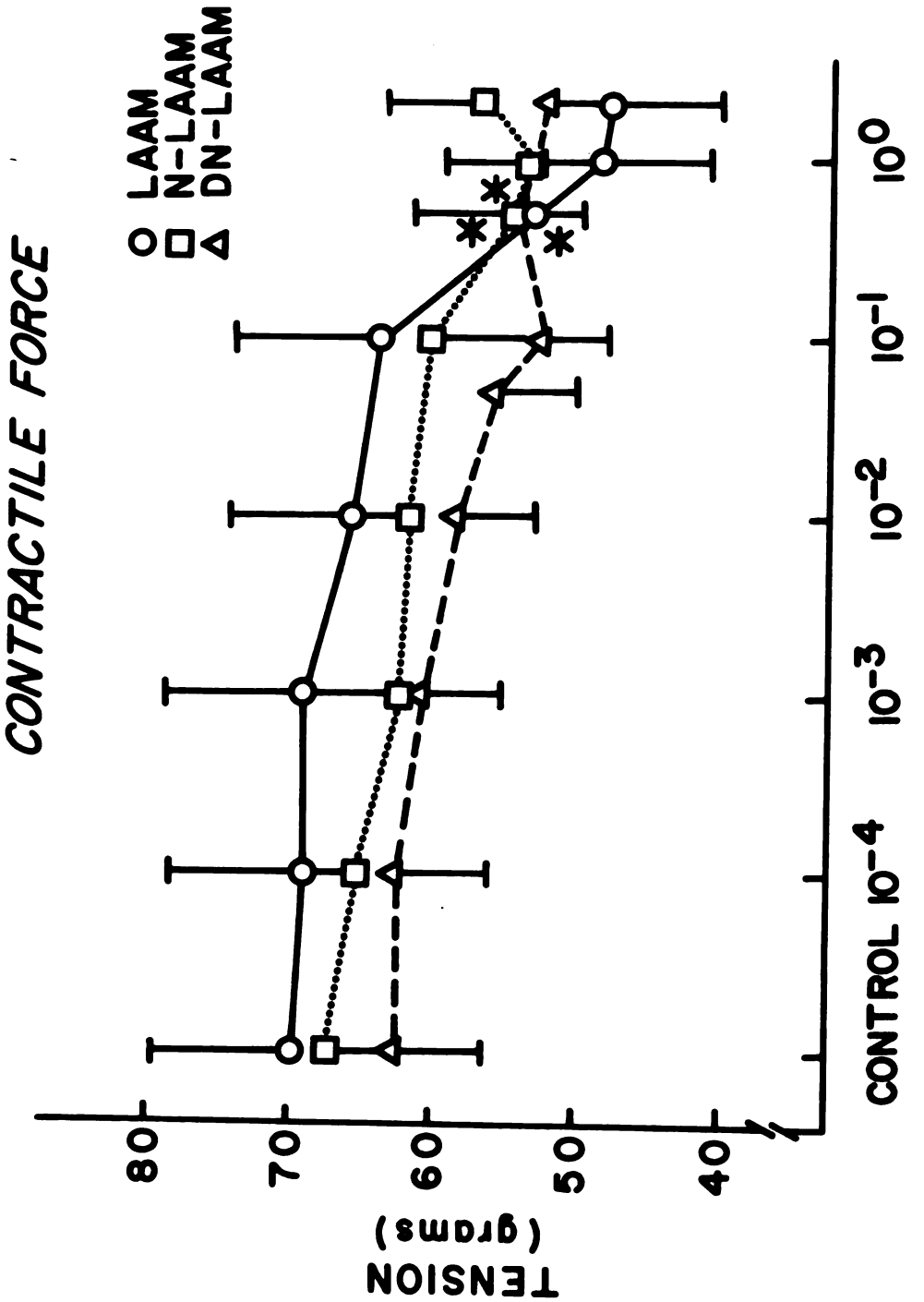
CONTRACTILE FORCE
LAAM
N-LAAM
DN-LAAM
TENSION
(grams)
80
70
60
50
40
CONTROL
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^{0}
DOSE
(μmoles/kg/7.0)

Figure 4

metabolites. The observations are summarized in Table 4. LAAM and dinor-LAAM had similar effects on the blood pressure response; the highest doses decreasing it to 31.4±4.1 and 35.9±7.2% of the control response, respectively. Nor-LAAM, at an equimolar dose, reduced the response to 10.3±3.1% of control. At the highest dose, all 3 compounds essentially eliminated the responses of HR and CF to BLCO. There was considerable variability in the threshold dose at which significant decreases in the BLCO responses were seen. However, nor-LAAM was consistently more potent in the inhibitory action than either LAAM or dinor-LAAM.

In order to investigate factors which may contribute to the BP response to LAAM, cardiac output and total peripheral resistance (TPR) were determined before and after cumulative doses of LAAM (1.4-5.5 mg/kg). The data are summarized in Table 5. At all doses, LAAM significantly decreased cardiac output, as well as BP and HR. After the highest dose of LAAM, 5.5 mg/kg, BP, HR, and cardiac output were decreased by 27, 55, and 55%, respectively. TPR was significantly increased after this dose of LAAM. The effects of LAAM on BP, TPR and cardiac output were then compared to the effects of the cardio-selective β-adrenergic blocking agent practolol. The site of action for practolol is the heart. Therefore, BP decreases observed following practolol administration are the result of decreases in cardiac output. As shown in Figure 5, LAAM produced a larger decrease in BP than practolol for any given decrease in cardiac output. TPR was increased by practolol and LAAM. The increases in TPR following practolol appeared to be somewhat larger than the increases in TPR following LAAM

TABLE 4

Effects of LAAM, nor-LAAM and dinor-LAAM on the Responses to Bilateral Carotid Occlusion in the Dog

Drug		Mean Blood Pressure Change (mmHg)	Heart Rate Change (Beats/min)	Contractile Force Change (gms)
LAAM	Control	+36.6±7.4[a]	+12.2±3.3	+12.4±4.6
	1.4×10^{1} µmoles/kg	+11.6±2.4[b]	+2.2±1.6[b]	+2.2±1.4[b]
	Threshold Dose[c]	7.0	7.0	1.4×10^{1}
nor-LAAM	Control	+50.0±4.5	+18.0±1.3	+9.0±2.2
	1.4×10^{1} µmoles/kg	+5.6±1.7[b]	-0.8±2.6[b]	-0.5±0.5[b]
	Threshold Dose	7.0×10^{-2}	7.0×10^{-1}	7.0×10^{-2}
dinor-LAAM	Control	+20.4±3.2	+12.8±2.6	+9.4±2.5
	1.4×10^{1} µmoles/kg	+11.9±3.5[b]	+0.4±2.6[b]	0.0±0.0[b]
	Threshold Dose	3.5	3.5	7.0×10^{-1}

[a] $\bar{X}$ ± S.E.M. For resting blood pressure, heart rate, and contractile force at the time of occlusion refer to Figures 2-4.

[b] Significantly less than control, $p<.05$.

[c] Dose in µmoles/kg.

TABLE 5

Effects of Cumulative Doses of LAAM and Practolol on Blood Pressure, Heart Rate, Cardiac Output and Total Peripheral Resistance in the Dog

Treatment	Mean Blood Pressure mmHg (% of control)	Heart Rate beats/min (% of control)	Cardiac Output liters/min (% of control)	Total Peripheral Resistance dyne·sec/cm^5 (% of control)
Control	123.6±7.2[a] (100)	150.5± 9.2[a] (100)	3.9± 0.3[a] (100)	2640±240[a] (100)
1.4 mg/kg LAAM	110.4±8.9[c] (89.3±7.2)	98.4±11.7[c] (65.3± 7.5)	2.9± 0.2[c] (74.4± 5.1)	3120±320 (118.1±12.1)
2.7 mg/kg LAAM	101.2±8.4[c] (81.8±7.1)	73.8± 7.2[c] (49.0± 4.8)	2.2± 0.2[c] (56.4± 5.1)	3840±480[c] (147.3±18.2)
5.5 mg/kg LAAM	94.4±7.4[c] (76.4±6.2)	62.8± 3.1[c] (41.7± 2.1)	1.9± 0.3[c] (48.7± 7.7)	4320±480[c] (164.5±18.2)
Control	125.0±0.7[b] (100)	161.4± 1.7[b] (100)	3.6± 0.5[b] (100)	2880±384[b] (100)
1.0 mg/kg Practolol	124.6±3.2 (99.7±2.6)	126.6± 7.8[c] (78.4± 7.8)	3.2± 0.3 (86.8± 7.1)	3200±288 (111.1±10.0)
2.5 mg/kg Practolol	118.9±4.7[c] (95.5±3.8)	124.0± 7.6[c] (76.8± 4.7)	2.8± 0.4[c] (76.7±10.9)	3520±512 (122.2±17.7)
5.0 mg/kg Practolol	115.8±1.5[c] (92.6±1.2)	108.8±14.5[c] (67.4± 8.9)	2.1± 0.35[c] (57.5± 9.5)	4640±640[c] (161.1±22.2)

[a] $\bar{X}$ ± S.E.M., N=5. [b] $\bar{X}$ ± S.E.M., N=3. [c] Significantly different from pre-drug control, $P<.05$.

Figure 5. Comparison of the effects of LAAM and practolol on blood pressure against their effects on cardiac output in the dog. The blood pressure decreases caused by 1.4, 2.7 and 5.5 mg/kg LAAM and 1.0, 2.5 and 5.0 mg/kg practolol on blood pressure are plotted as a function of the decrease in cardiac output produced by that same dose. Each point represents the $\bar{X}\pm$S.E.M., N=3.

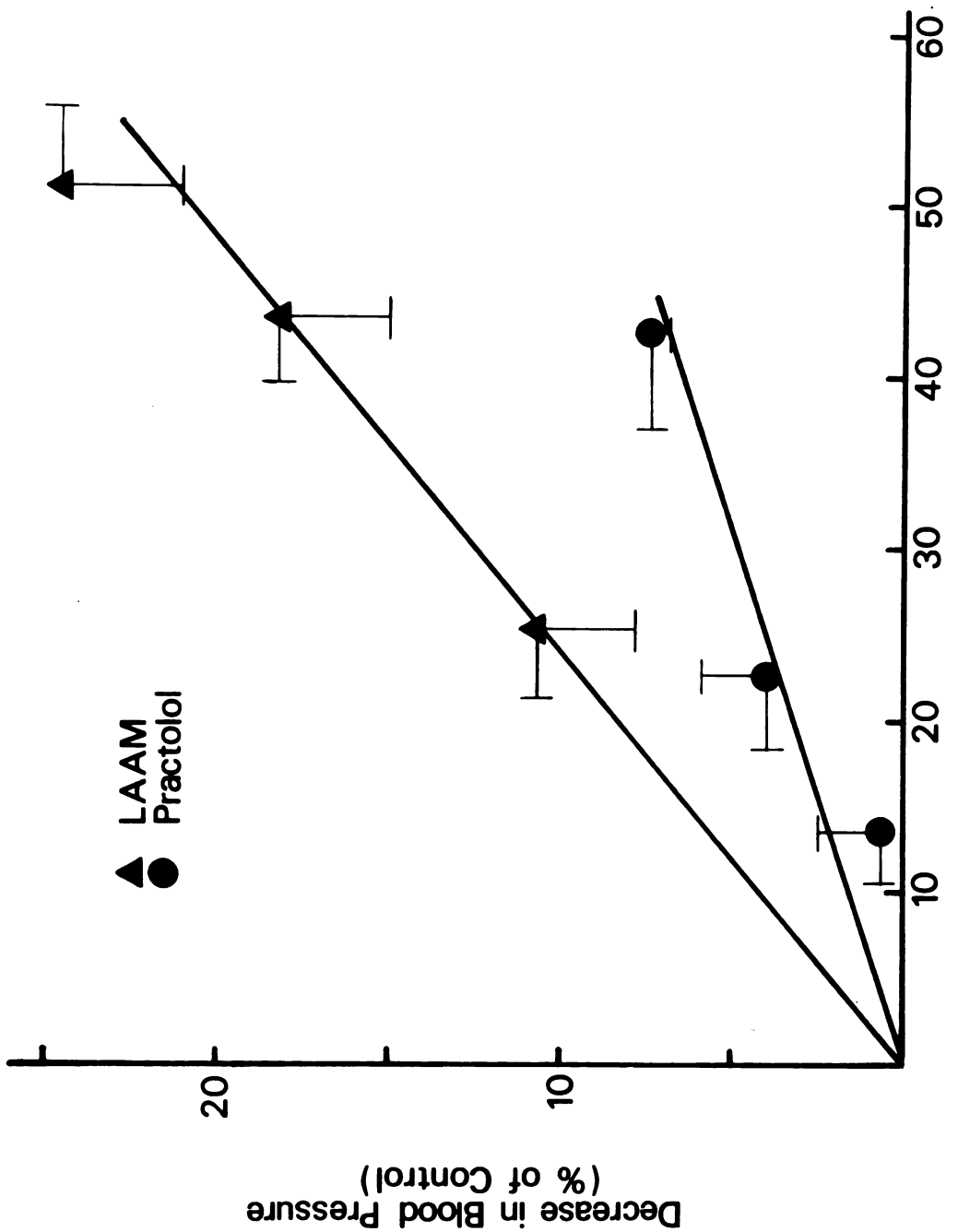
LAAM
Practolol
Decrease in Blood Pressure
(% of Control)
Decrease in Cardiac Output
(% of Control)
10
20
30
40
50
60
10
20

Figure 5

for comparable decreases in cardiac output. The data suggest that the BP response to LAAM is the result of vascular changes as well as changes in cardiac output.

The cardiovascular responses to LAAM were examined in anesthetized cats to determine if the in vivo cardiovascular effects of LAAM observed in the dog were unique to that species. This study also was a preliminary experiment for later studies in which the cat nictitating membrane preparation was used to examine the effects of LAAM on the peripheral sympathetic nervous system (Table 6). BP, HR, and CF were decreased significantly after a dose of 1.4 mg/kg in the cat. After the highest dose of LAAM given, BP, HR, and CF all were decreased to about 60% of their respective control values. These data indicate that the minimum doses which significantly decrease BP, HR, and CF in cats and dogs are the same, 1.4 mg/kg.

B. The Autonomic Nervous System and the Cardiovascular Actions of LAAM

1. In Vivo Experiments

BP and HR were simultaneously decreased by LAAM. The responses of BP, HR, and CF to BLCO, also are decreased by LAAM. Furthermore, the HR response to LAAM is antagonized by atropine (Stickney, 1978a). These data suggest that LAAM may act on the autonomic nervous system to produce its cardiovascular actions. Therefore, experiments were performed to evaluate the contribution of the effects of LAAM on the sympathetic and parasympathetic nervous systems to the cardiovascular actions of LAAM. The effects of LAAM were examined in vagotomized (VAGOT), sympathectomized (SYMX) and sympathectomized and vagotomized

TABLE 6

Effects of Cumulative Doses of LAAM on Blood Pressure, Heart Rate and Contractile Force in the Cat

LAAM Dose (mg/kg)	Mean Blood Pressure (mmHg)	Heart Rate (bpm)	Contractile Force (gms)
Control	99.3±8.4[a]	199.3± 8.8[a]	40.0±3.5[a]
$2.7x10^{-4}$	102.3±7.9	200.7± 7.9	40.3±3.3
$2.7x10^{-3}$	97.3±5.2	200.7± 9.8	39.6±3.9
$2.7x10^{-2}$	99.0±9.5	197.3± 7.4	36.2±2.7
$2.7x10^{-1}$	102.7±5.4	194.6± 7.9	33.3±1.7
1.4	74.3±4.7[b]	173.3±13.5[b]	26.3±4.1[b]
2.7	65.6±1.3[b]	156.7±11.6[b]	23.5±3.6[b]
5.5	59.3±2.8[b]	130.7± 5.9[b]	22.5±5.2[b]

[a] $\bar{X}$ ± S.E.M., N=3.

[b] Significantly less than pre-LAAM control, $p<.05$.

(SYMX + VAGOT) animals. The effects of LAAM on BP in these, as well as intact animals, are shown in Figure 6.

LAAM significantly decreased BP regardless of the type of autonomic lesion present. However, the minimum dose required for significant decreases varied depending on the lesion. VAGOT alone did not appear to alter significantly the effects of LAAM on BP. The lowest dose which significantly decreased BP was 1.4 mg/kg in both intact and VAGOT animals. Furthermore, the magnitudes of the responses were similar. SYMX and SYMX + VAGOT, unlike VAGOT, significantly altered the minimum dose required for the depressor response. In these groups significant BP changes were observed only after 5.5 mg/kg, the highest dose given. SYMX and SYMX + VAGOT appeared to reduce the absolute decrease as well as the percent decrease in mean BP relative to that observed in intact animals.

Significant decreases in HR also were observed in all lesion groups after LAAM (Figure 7). No difference in the minimum dose required to produce bradycardia was observed, 1.4 mg/kg in all groups. However, the type of lesion appeared to influence the magnitude of the response. The heart rate response to LAAM in SYMX and SYMX + VAGOT animals was significantly less than that observed in intact animals. The HR response to LAAM in VAGOT animals appeared to be less, but was not significantly different from, the HR response to LAAM in intact animals.

As shown in Figure 8, CF was decreased in all 3 lesion groups. However, the responses were not significantly different from those obtained in intact animals. The minimum dose required to produce

Figure 6. Cumulative dose-response curves for the effects of LAAM on mean arterial blood pressure in intact, vagotomized (VAGOT), sympathectomized (SYMX) and sympathectomized + vagotomized (SYMX + VAGOT) dogs. Each dose represents the total amount of drug given at the time the measurements were made. Control values represent pre-LAAM values in each case. In the upper right hand corner is the key for the symbols used in the curves. The asterisks (*) denote the threshold dose for significant decreases from respective control values, $p<.05$. Each data point represents the mean of 5 replications; the vertical lines represent the S.E.M.

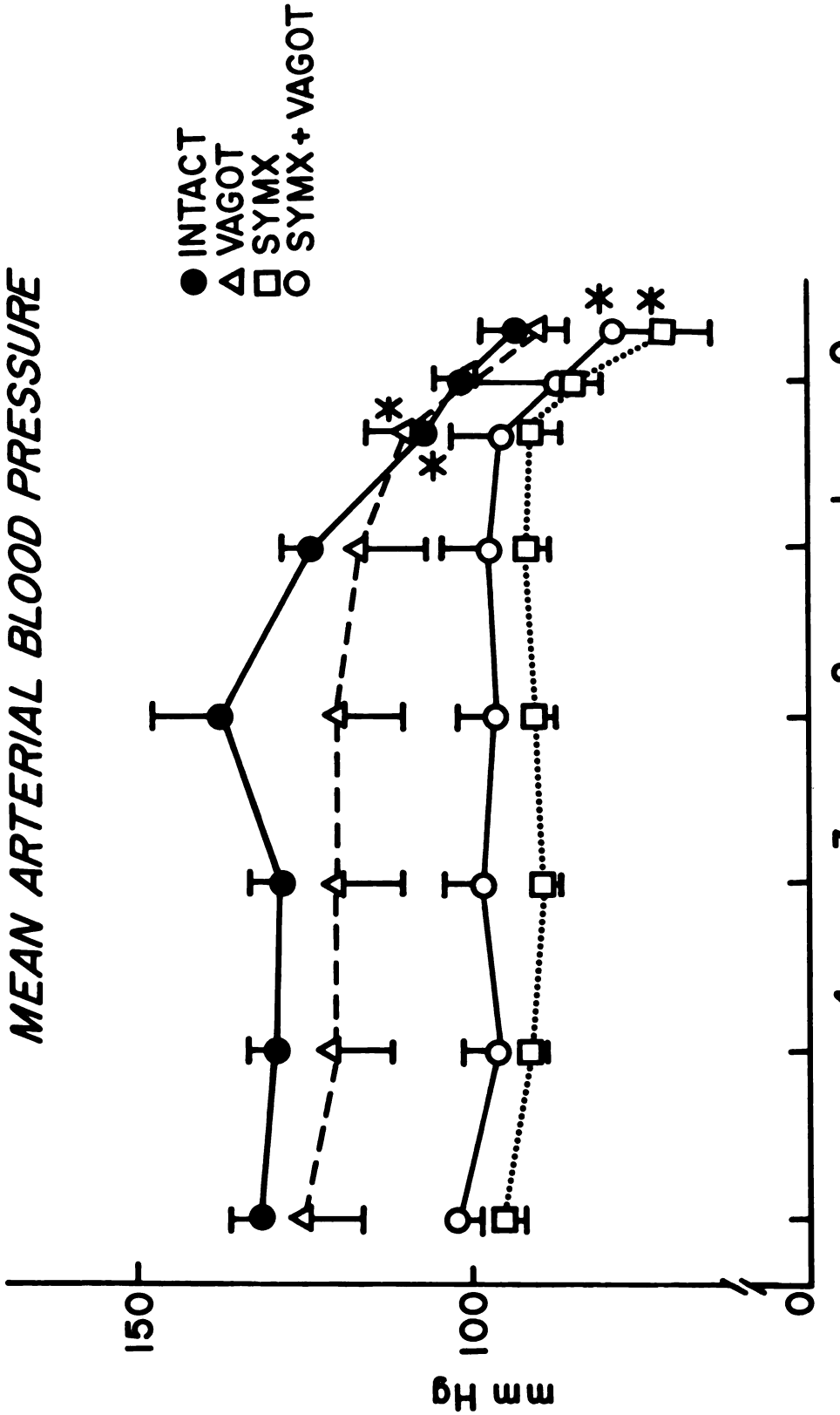
MEAN ARTERIAL BLOOD PRESSURE
INTACT
VAGOT
SYMX
SYMX + VAGOT
150
100
0
mm Hg
CONTROL
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^{0}
DOSE OF LAAM
(μmoles/kg/7.0)

Figure 6

Figure 7. Cumulative dose-response curves for the effects of LAAM on heart rate in intact, VAGOT, SYMX and SYMX + VAGOT dogs. Each dose represents the total amount of drug given at the time the measurements were made. Control values represent pre-LAAM values in each case. In the upper right hand corner is the key for the symbols used in the curves. The asterisks (*) denote the threshold doses for significant decreases from the respective control values, $p<.05$. Each data point represents the mean of 5 replications; the vertical lines represent the S.E.M.

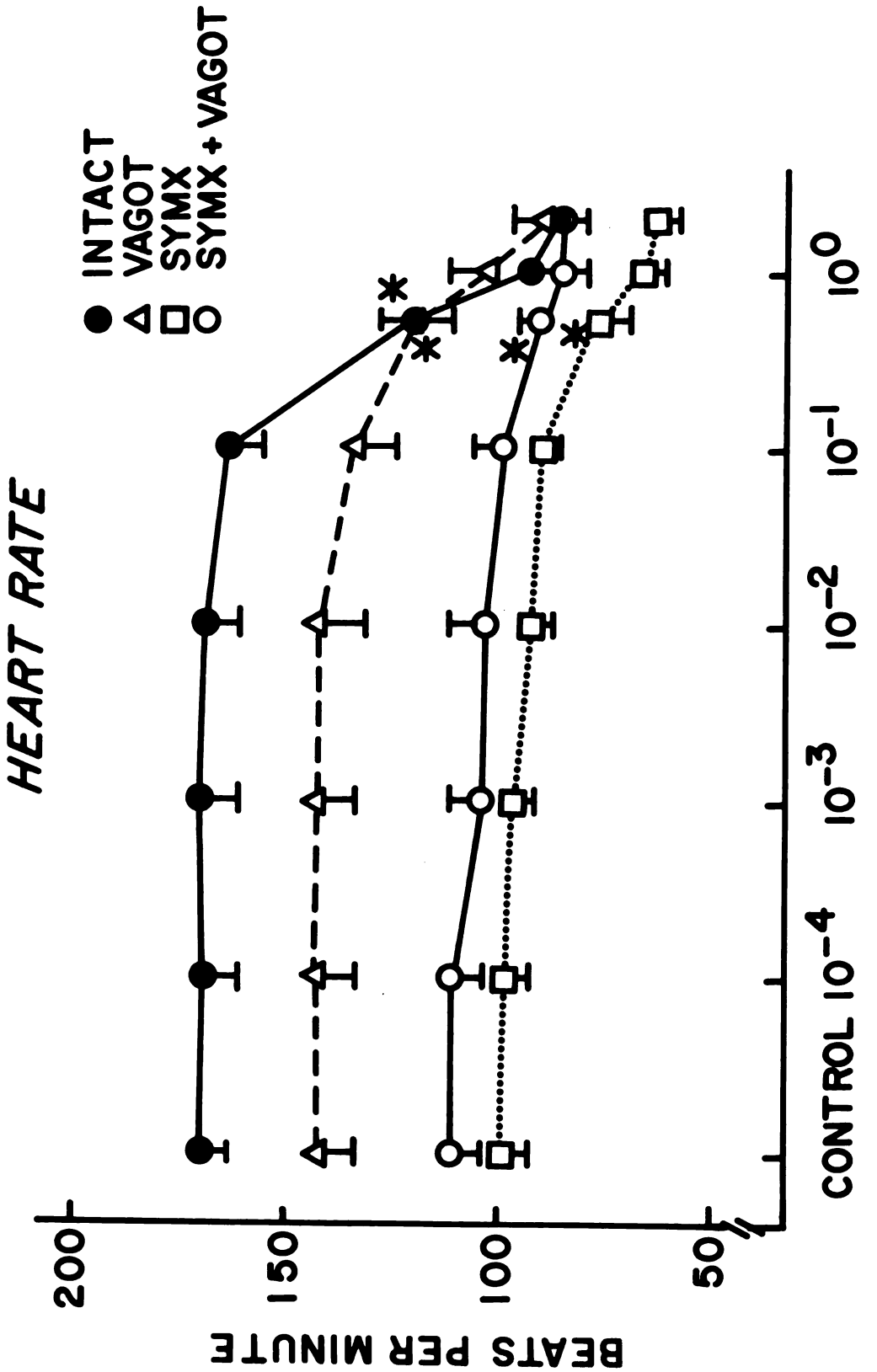
HEART RATE
INTACT
VAGOT
SYMX
SYMX + VAGOT
BEATS PER MINUTE
200
150
100
50
CONTROL
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^{0}
DOSE OF LAAM
(μ moles / kg / 7.0)

Figure 7

Figure 8. Cumulative dose-response curves for the effects of LAAM on right ventricular contractile force in intact, VAGOT, SYMX and SYMX + VAGOT dogs. Each dose represents the total amount of drug given at the time the measurements were made. Control values represent pre-LAAM values in each case. In the upper right hand corner is the key for the symbols used in the curves. The asterisks (*) denote the threshold doses for significant decreases from the respective control values, $p<.05$. Each data point represents the mean of 5 replications; the vertical lines represent the S.E.M.

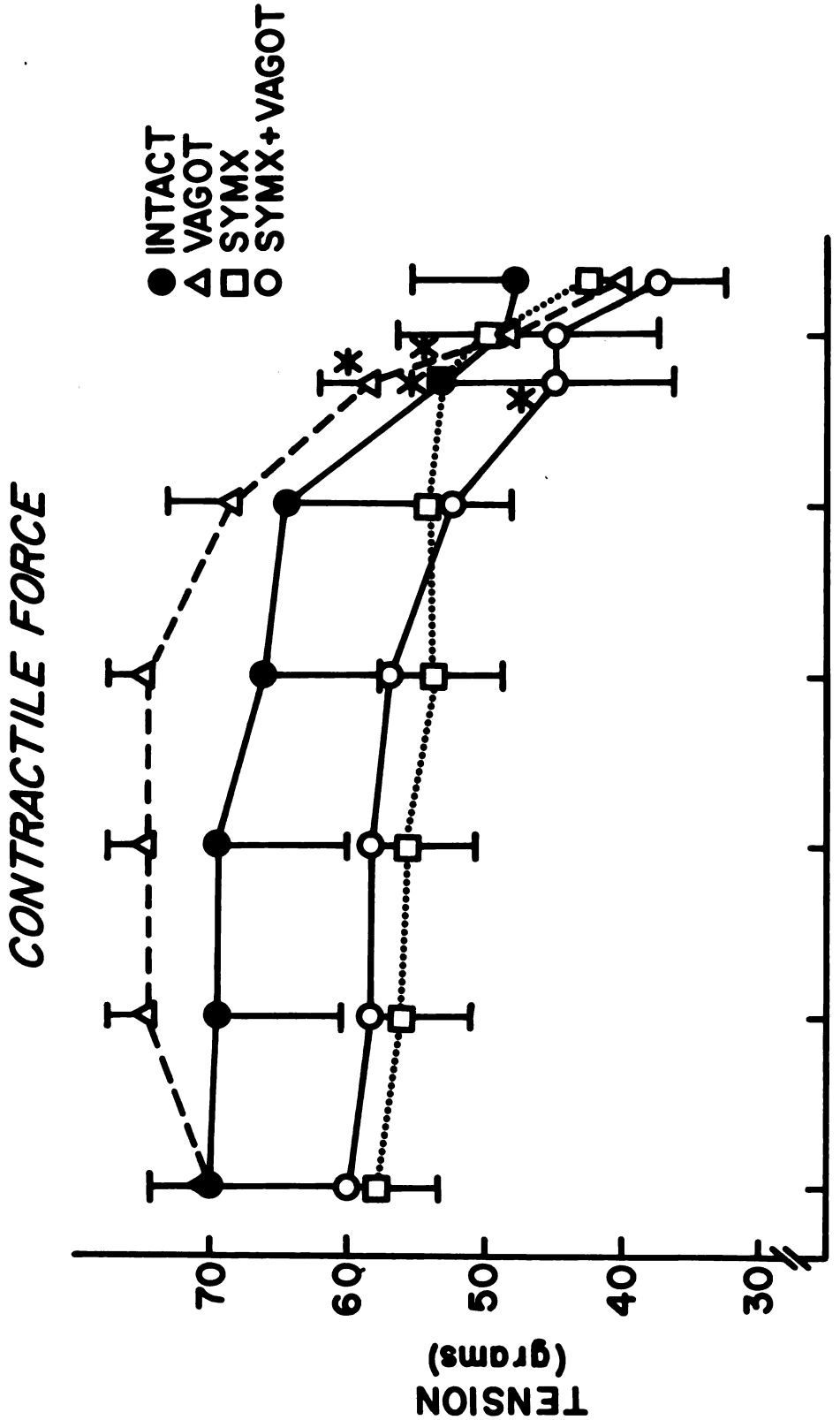
CONTRACTILE FORCE
INTACT
VAGOT
SYMX
SYMX+VAGOT
TENSION
(grams)
70
60
50
40
30
CONTROL
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^{0}
DOSE OF LAAM
(μ moles/kg/7.0)

Figure 8

the effect was the same in all groups and the lesions appeared to have minimal effects on the magnitude of the decrease in CF.

The responses to BLCO were monitored in SYMX, SYMX + VAGOT, and VAGOT animals. However, in sympathectomized animals, the responses to BLCO were very small compared to intact animals (Table 4) and the effects of LAAM on these responses will not be presented. Table 7 shows the effects of LAAM on responses to BLCO in VAGOT animals. The minimum dose required for significant decreases in the BP response to BLCO was the same as that observed in the intact animals, 2.7 mg/kg (Table 4). The minimum dose required for significant decreases in the HR response to BLCO was higher in vagotomized dogs, 5.5 mg/kg. This may indicate that vagal influence on the heart is affected by LAAM. However, VAGOT did not change the minimum dose of LAAM required to produce a significant decrease in HR or the magnitude of the HR response. The minimum dose of LAAM required for significant decreases in the CF response to BLCO was 2.7 mg/kg in VAGOT animals as compared to 5.5 mg/kg in intact animals. The magnitudes of the CF effects at the highest dose given, 5.5 mg/kg, appeared similar in all lesion groups.

2. In Vitro Enzyme Studies

The HR response to LAAM was not significantly affected by vagotomy yet atropine attenuates the effects of LAAM on HR (Stickney, 1978a). In vitro studies have indicated that LAAM may decrease HR, in part, via cholinesterase inhibition (Stickney, 1978a). This led to the examination of the anticholinesterase activity of LAAM and its metabolites. The effects of LAAM and its active metabolites, nor-LAAM and dinor-LAAM, on heart homogenate and plasma cholinesterase activity

TABLE 7

Effects of LAAM on Bilateral Carotid Occlusion Responses in Vagotomized Dogs

Dose of LAAM (μmoles/kg)	Mean Blood Pressure Change (mmHg)	Heart Rate Change (bpm)	Contractile Force Change (gms)
Control	+32.2± 4.6[a]	+9.2±3.2	+14.5±5.5
Postvagotomy	+73.2± 8.6	+17.4±5.3	+29.6±7.8
$7x10^{-4}$	+72.2± 9.8	+17.6±3.6	+31.8±6.5
$7x10^{-3}$	+77.2± 7.7	+15.4±3.0	+34.5±6.4
$7x10^{-2}$	+79.4±10.2	+14.6±2.3	+31.9±6.0
$7x10^{-1}$	+74.4±10.8	+15.4±2.6	+28.4±5.8
3.5	+65.8±11.6	+13.2±2.9	+23.0±7.0
7.0	+44.6± 7.9[b]	+10.8±2.8	+12.5±5.1[b]
$1.4x10^{1}$	+24.6± 2.4[b]	+6.4±1.6[b]	5.0±1.6[b]

[a] $\bar{X}$ ± S.E.M. For resting blood pressure, heart rate and contractile force at the time of occlusion, refer to Figures 6-8.

[b] Significantly less than postvagotomy, pre-LAAM control.

are shown in Figure 9. LAAM ($1x10^{-4}M$) decreased the rate of hydrolysis of acetylcholine by heart homogenates and plasma to 82.11±4.26 and 43.43±4.37 percent of control, respectively. Nor-LAAM and dinor-LAAM (each $1x10^{-4}M$) also significantly decreased heart homogenate and plasma enzyme activities. The effects of all three compounds were much more pronounced on plasma enzyme activity.

The effects of LAAM, nor-LAAM and dinor-LAAM on the activities of acetylcholinesterase and butyrylcholinesterase also were examined (Figure 10). LAAM ($1x10^{-4}M$) decreased the activity of acetylcholinesterase and butyrylcholinesterase preparations to 86.16±4.21 and 31.66±6.15% of control, respectively. As was the case in plasma and heart homogenates, the metabolites nor-LAAM and dinor-LAAM exhibited anticholinesterase activity similar to the parent compound in both acetylcholinesterase and butyrylcholinesterase preparations (Figure 10). LAAM and its metabolites appeared to be less potent inhibitors of the acetylcholineterase than of the butyrylcholinesterase.

The anticholinesterase activity of LAAM was greater in preparations containing plasma enzyme or butyrylcholinesterase. The effect of LAAM on these two enzymes was examined further. Figure 11 shows the concentration-effect curves obtained. In the case of plasma enzyme, maximum inhibition of approximately 75% was observed at a concentration of $1x10^{-3}M$ LAAM. The IC_{50} for plasma was $2x10^{-4}M$. In the butyrylcholinesterase preparation, 100% inhibition was observed at approximately $1x10^{-3}M$. The IC_{50} in this preparation was $6x10^{-5}M$. The concentration-effect curves appear to be parallel.

Figure 9. Effects of LAAM and its major metabolites, nor-LAAM and dinor-LAAM, on cholinesterase activity in preparations of guinea-pig heart homogenates and heparinized guinea-pig plasma *in vitro*. In all cases treated rates of acetylcholine hydrolysis were significantly less than control rates of hydrolysis, $p<.05$. Each bar represents the mean of at least 3 replications. Vertical lines represent S.E.M. Control enzyme activities were ($\bar{X}\pm$S.E.M.): heart homogenate, 1.07± .21 nmoles acetylcholine/min/mg protein; plasma 171.1±17.3 nmoles acetylcholine/min/ml plasma.

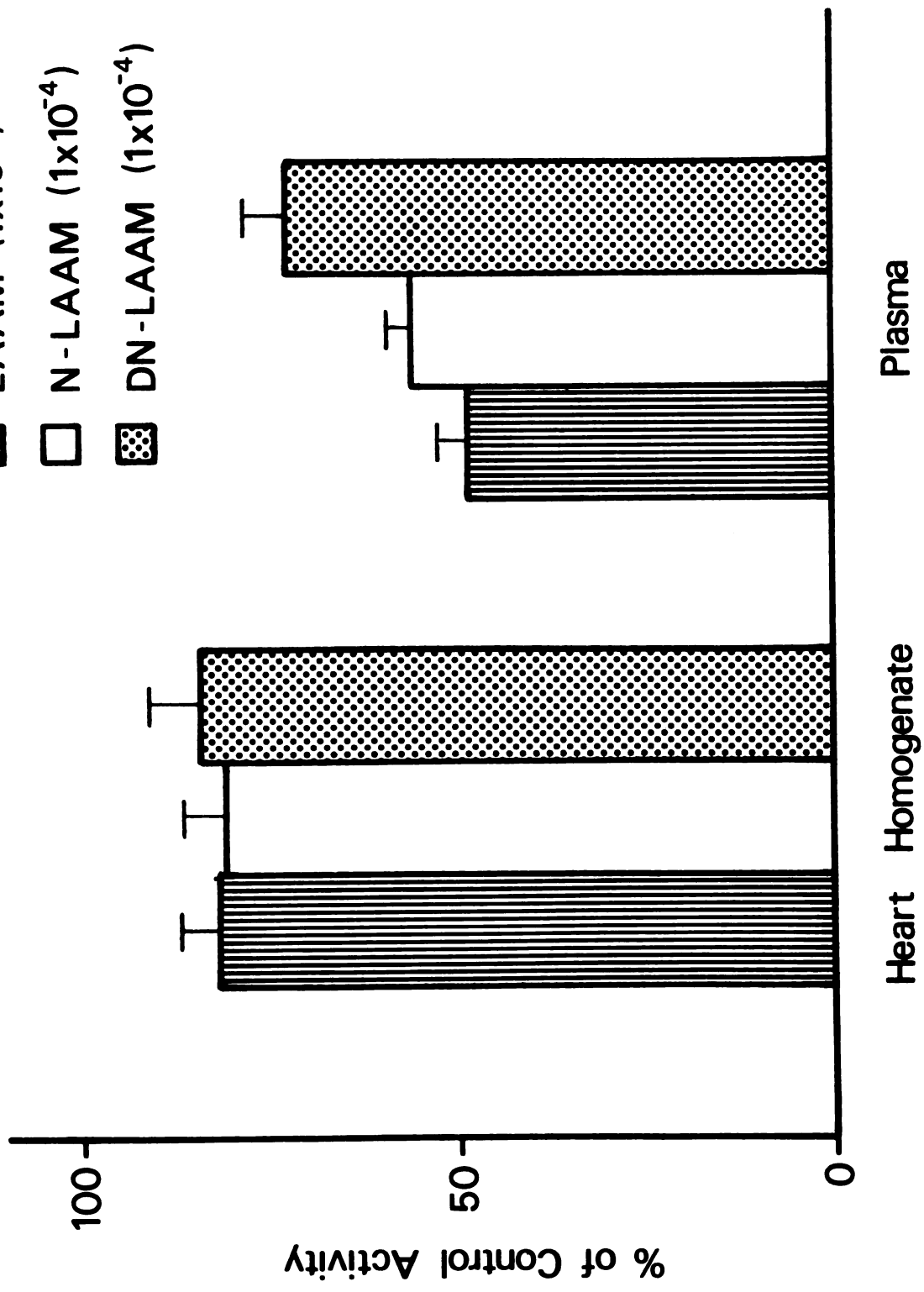
LAAM (1x10^{-4})
N-LAAM (1x10^{-4})
DN-LAAM (1x10^{-4})
% of Control Activity
100
50
0
Heart Homogenate
Plasma

Figure 9

Figure 10. Effects of LAAM and its major metabolites, nor-LAAM and dinor-LAAM, on cholinesterase activity in preparations of purified acetylcholinesterase and purified butyrylcholinesterase *in vitro*. In all cases treated rates of hydrolysis were significantly less than control rates of acetylcholine hydrolysis, $p<.05$. Each bar represents the mean of at least 3 replications. Vertical lines represent S.E.M. Control enzyme activities were: Acetylcholinesterase, 0.27 μmoles/mg protein/min; Butyrylcholinesterase, 2.23±0.03 μmoles/mg protein/min.

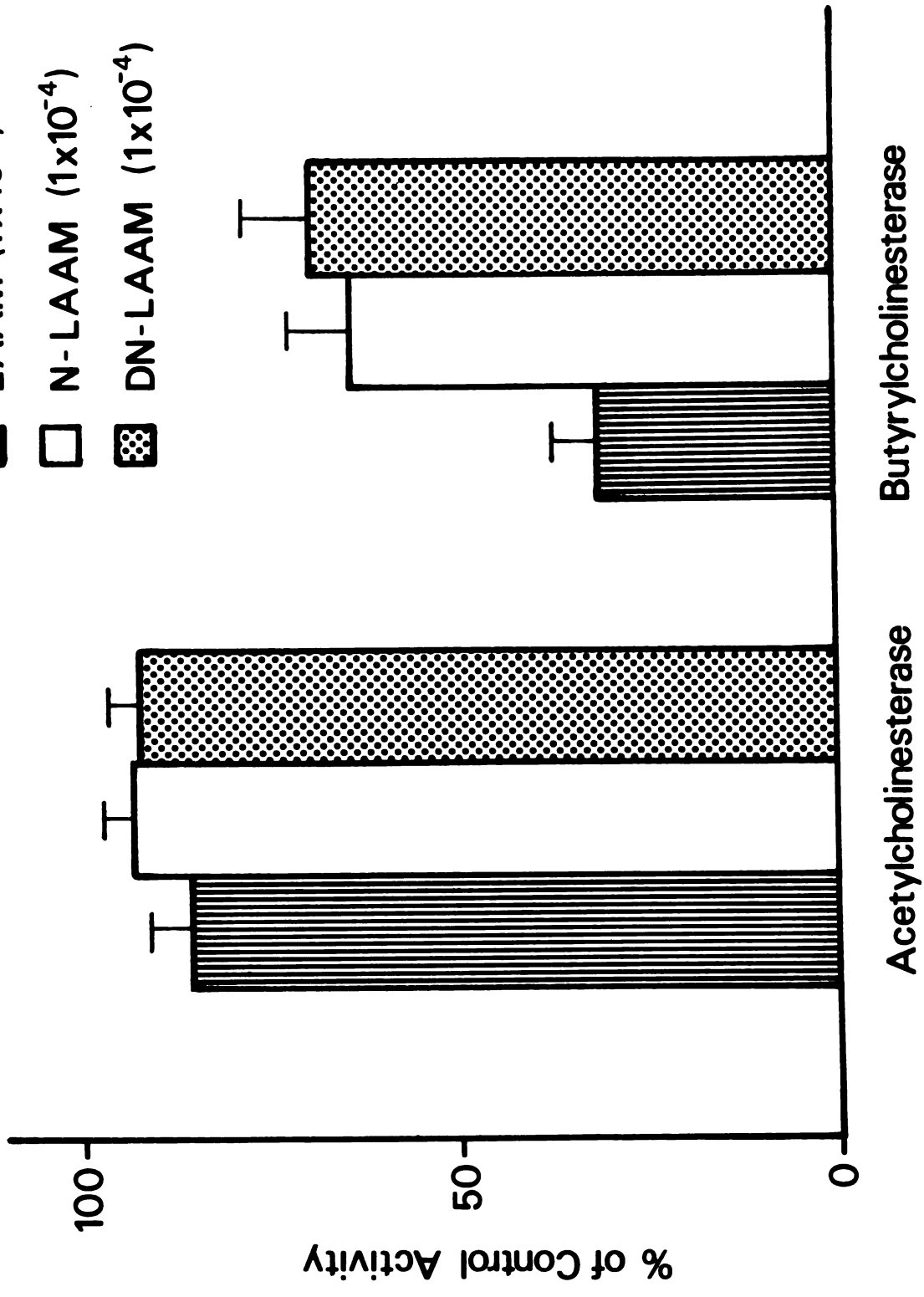
LAAM (1x10^{-4})
N-LAAM (1x10^{-4})
DN-LAAM (1x10^{-4})
% of Control Activity
100
50
0
Acetylcholinesterase
Butyrylcholinesterase

Figure 10

Figure 11. Concentration-effect curves for the inhibitory action of LAAM in preparations of guinea-pig plasma (▲) and purified butyrylcholinesterase (●) in vitro. Each point represents the mean of at least 3 replications. Vertical lines represent S.E.M.

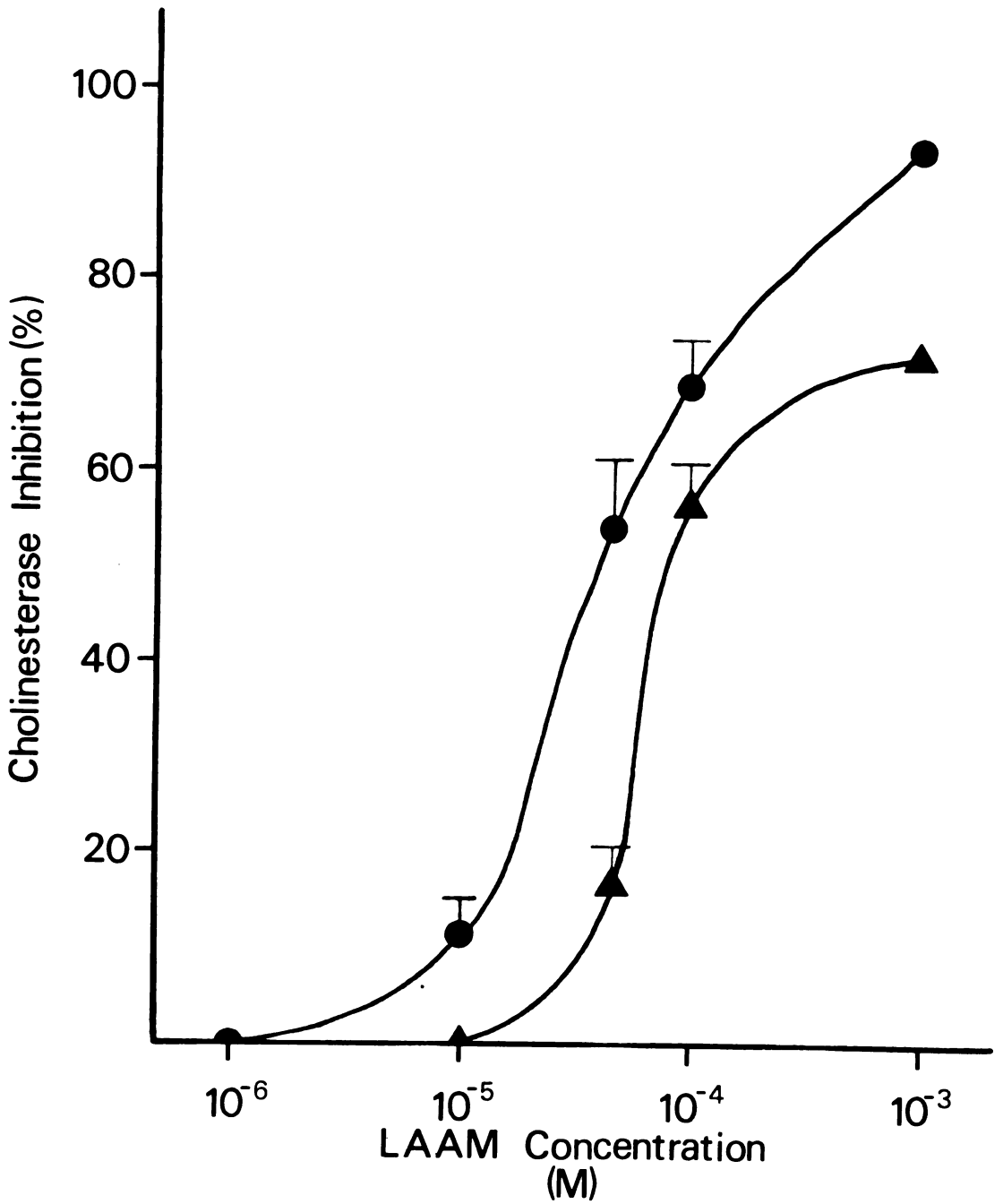
100
80
60
40
20
Cholinesterase Inhibition (%)
10^{-6}
10^{-5}
10^{-4}
10^{-3}
LAAM Concentration
(M)

Figure 11

In addition to examining the effects of LAAM and its metabolites on the various cholinesterases, the anticholinesterase activity of LAAM was compared to that of other narcotics and to physostigmine, a known cholinesterase inhibitor. This comparison was made in both heart homogenates and plasma. The data are summarized in Table 8. Methadone, like LAAM, produced a larger decrease in plasma enzyme activity than in heart homogenate enzyme activity. Morphine, on the other hand, had no significant effect on either the heart or plasma enzyme activities at equimolar concentrations (Table 8). Physostigmine differed in two respects from the narcotics studied: (1) it was more potent, and (2) it showed similar degrees of anticholinesterase activity in both the heart homogenates and the plasma.

3. Isolated Atria Studies

Experiments using the isolated guinea pig right atria preparation had suggested that LAAM enhanced the negative chronotropic activity of methacholine by inhibiting cholinesterase (Stickney, 1978a). However, in vitro enzyme studies suggest that only high concentrations of LAAM inhibit cholinesterase. Therefore, the effect of LAAM on the negative chronotropic activity of another cholinergic agonist, bethanechol, which is not hydrolyzed by cholinesterase, was examined. Bethanechol produced a dose related decrease in the spontaneous rate of beating of isolated guinea pig right atria (Figure 12). At $1x10^{-6}$M, bethanechol produced a 24.57±5.37% decrease in the rate of beating and a 97.22±2.73% decrease at $5x10^{-5}$M. In the presence of $1x10^{-7}$M physostigmine, $1x10^{-6}$M bethanechol produced a 20.38±3.34% decrease in the spontaneous rate of beating. This decrease is not significantly

TABLE 8

Comparison of the Effects of Several Drugs on the Cholinesterase Activity in Guinea-pig Heart Homogenates and Heparinized Guinea-pig Plasma Samples In Vitro

Drug	Concentration (M)	% of Control Activity	
		Heart Homogenate	Plasma
LAAM	$1x10^{-4}$	$82.11\pm4.26^{a,b}$ N=6	43.43 ± 4.37^{b} N=8
Methadone	$1x10^{-4}$	91.22 ± 5.12^{b} N=3	53.80 ± 9.90^{b} N=3
Morphine	$1x10^{-4}$	101.01 ± 4.69 N=3	97.41 ± 6.7 N=3
Physostigmine	$1x10^{-7}$	43.74 ± 7.63^{b} N=3	61.82 ± 1.16^{b} N=3

[a]Mean ± S.E.M. For control enzyme activites see Figure 9.

[b]Significantly different from control, $p<.05$.

Figure 12. Concentration-effect curves for bethanechol (●), bethanechol in the presence of $1x10^{-7}$M physostigmine (■), and bethanechol in the presence of $5x10^{-6}$M LAAM (▲) in the isolated guinea pig right atrium preparation. All atria ceased beating after $5x10^{-5}$M bethanechol in the presence of either physostigmine or LAAM. Only 1 atrium continued to beat at this concentration of bethanechol alone. Each point represents the mean of at least 5 replications. Vertical lines represent S.E.M. BPM indicates beats per minute.

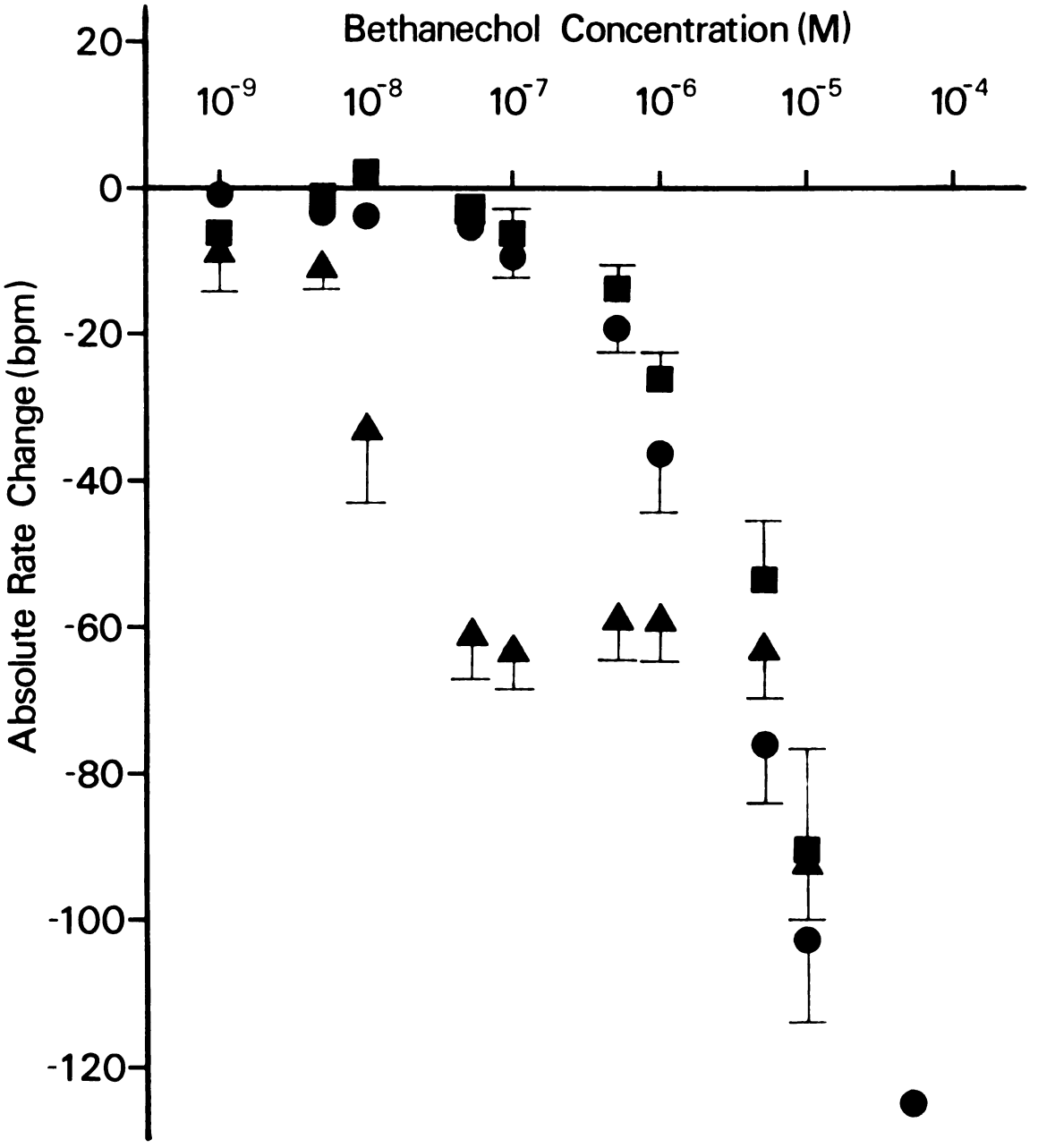
Bethanechol Concentration (M)
10-9
10-8
10-7
10-6
10-5
10-4
20
0
-20
-40
-60
-80
-100
-120
Absolute Rate Change (bpm)

Figure 12

different from the decrease observed in the presence of bethanechol alone, $p>0.05$. LAAM, $5x10^{-6}M$, significantly potentiated the effect of low concentrations of bethanechol on the guinea pig right atria preparation. In the presence of LAAM, $5x10^{-6}M$ bethanechol produced a 55.96±5.75% decrease in the rate of beating as opposed to a 19.78±2.12% decrease in control experiments, $p<0.05$.

C. The Involvement of Opiate Binding Sites in the Mechanisms for the Cardiovascular Effects of LAAM and nor-LAAM

1. General Mechanisms of Action

The cardiovascular actions of several narcotics involve the interaction of these agents with opiate binding sites (Laubie et al., 1974; Daskalopoulos et al., 1975). Experiments were performed to determine if an interaction of LAAM with opiate binding sites contributed to the cardiovascular actions of LAAM and nor-LAAM. The effects of pretreatment with the narcotic antagonist naltrexone, 300 µg/kg, s.c., on the BP, HR and CF responses to injections of LAAM (2.7 mg/kg) and nor-LAAM (0.26 mg/kg) in dogs are shown in Table 9. Also shown are the effects of LAAM and nor-LAAM injections without naltrexone pretreatment. Table 10 shows the responses to BLCO in these experiments. LAAM (2.7 mg/kg) alone caused a significant decrease in BP and HR, reducing them to 80.3±8.1 and 50.6±2.4% of control, respectively. This dose of LAAM also significantly decreased the responses of BP and HR to BLCO. LAAM decreased CF to 67.0±4.8% of control and decreased the CF response to BLCO. Nor-LAAM (0.26 mg/kg) alone caused significant decreases in BP and HR to 81.7±11.4 and 68.7±14.9% of control, respectively. The BP and HR responses to BLCO also were reduced. Nor-LAAM at this dose did

TABLE 9

Effects of Naltrexone Pretreatment (300 μg/kg) on the Cardiovascular Responses to Injections of LAAM and nor-LAAM in the Dog

Treatment	Pretreatment	Mean Blood Pressure After Treatment	Heart Rate After Treatment	Contractile Force After Treatment
LAAM 2.7 mg/kg	None	80.3±8.1[b,c]	50.6±2.4[b,c]	67.0±4.8[b,c]
	Naltrexone[a]	97.8±5.1[d]	87.8±1.8[c,d]	86.5±11.5[d]
nor-LAAM 0.26 mg/kg	None	81.7±11.4[b,c]	68.7±14.9[b,c]	85.9±12.3[b]
	Naltrexone[a]	95.1± 4.0[d]	96.5± 1.2[d]	96.3± 5.0

[a] 300 μg/kg s.c. 35 min before the narcotic agonist injection.

[b] % of control, $\bar{X}$ ± S.E.M., N=3. Combined control values for all animals were: mean blood pressure (mmHg), 127.6±4.5; heart rate (beats/min) 154.8±4.3; contractile force (gms), 67.2±4.1.

[c] Significantly less than pre-LAAM control, $p<.05$.

[d] Significantly different from effect of LAAM alone.

TABLE 10

Effects of LAAM and nor-LAAM on the Responses to Bilateral Carotid Occlusion in Naltrexone (300 μg/kg) Pretreated Dogs

Treatment	Pretreatment	Bilateral Carotid Occlusion Responses		
		Mean Blood Pressure Change After Treatment	Heart Rate Change After Treatment	Contractile Force Change After Treatment
LAAM 2.7 mg/kg	None	30.7±10.1[c]	33.0± 7.0[c]	6.0± 6.0[c]
	Naltrexone[a]	91.7±16.0[d]	72.5±15.0[c,d]	74.6±20.9[d]
nor-LAAM 0.26 mg/kg	None	37.3± 8.2[c]	57.1±13.3[c]	49.6± 9.6[c]
	Naltrexone[a]	103.3±27.7[d]	67.2±41.8	72.4±29.3

[a] 300 μg/kg s.c. 35 min before the narcotic agonist injection.

[b] % of control, $\bar{X}$ ± S.E.M., N=3. Control responses to occlusion were: mean blood pressure (mmHg) 34.2±3.1; heart rate (beats/min), +11.3±1.1; contractile force (gms) 8.3±0.9.

[c] Significantly less than pre-LAAM control, $p<.05$.

[d] Significantly different from effect of LAAM alone, $p<.05$.

not significantly decrease CF but did reduce the CF response to BLCO by 50%. The fact that 0.26 mg/kg nor-LAAM causes effects on BP and HR similar to those produced by 2.7 mg/kg LAAM is in agreement with earlier data suggesting that nor-LAAM is about ten times more potent in producing these effects (Figures 2 and 3). The BLCO data also support this contention.

Naltrexone pretreatment (300 μg/kg) completely antagonized the effects of LAAM and nor-LAAM on BP as well as the effects of LAAM on the BP responses to BLCO (Tables 9 and 10). The effects of nor-LAAM (0.26 mg/kg) on HR and the HR response to BLCO were antagonized completely by naltrexone pretreatment (300 μg/kg) whereas the effects of LAAM (2.7 mg/kg) on these parameters were attenuated. LAAM decreased HR to 87.1±1.8% of control after naltrexone (300 μg/kg) as opposed to 50.6±2.4% of control without naltrexone. The effects of LAAM on CF were reduced significantly from a decrease of 33.0±4.8% of control after LAAM alone to 13.5±11.5% of control in naltrexone pretreated (300 μg/kg) animals. The effect of LAAM and nor-LAAM on the CF responses to BLCO were blocked completely by naltrexone pretreatment. These data suggest that the primary mechanism for the effects of LAAM and nor-LAAM on BP and the BP response to BLCO, at the doses used, involves an interaction of these drugs with opiate binding sites. Nor-LAAM (0.26 mg/kg) appears to produce its effects on HR primarily by interacting with opiate binding sites. In contrast to this, the effects of LAAM (2.7 mg/kg) on HR and the HR response to BLCO involve other mechanisms in addition to interactions of LAAM with opiate binding sites. The

mechanisms for the effects of LAAM (2.7 mg/kg) on CF and of LAAM and nor-LAAM on the CF response to BLCO appear to involve drug interactions with opiate binding sites.

The cardiovascular response to LAAM was examined in animals pretreated with lower doses of naltrexone (5 μg/kg). The purpose of these experiments was to study the effects of LAAM on the cardiovascular system in animals where the antagonism of the BP response could be overcome. Table 11 shows that 5 μg/kg naltrexone completely antagonizes the effect of 1.4 mg/kg LAAM on BP. However, 5.5 mg/kg LAAM, did significantly decrease BP in the presence of 5 μg/kg. These data suggest that if sufficiently low doses of antagonist are used, LAAM can overcome the blockade of the BP response by naltrexone. These results also suggest that the inability of 300 μg/kg naltrexone to completely antagonize the HR response to LAAM is not because the dose of antagonist was not large enough.

2. Effects of LAAM on the Peripheral Sympathetic Nervous System

The experiments in SYMX animals had suggested that LAAM might act on the sympathetic nervous system to produce its cardiovascular effects. The cat nictitating membrane preparation was used to study the effects of LAAM on the peripheral sympathetic nervous system and the relationship of these effects to the cardiovascular actions of LAAM. The effect of atropine pretreatment was also studied in the experiments. Results described earlier suggested that LAAM was acting through muscarinic receptors and there is evidence for the modulation of peripheral sympathetic neurotransmission by presynaptic muscarinic receptor activation (La Vallee et al., 1978). Figure 13 shows the effects of

TABLE 11

Effects of Naltrexone Pretreatment (5 μg/kg) on the Cardiovascular Responses to Several Doses of LAAM in the Dog

Dose of LAAM	Pretreatment	Mean Blood Pressure After LAAM	Heart Rate After LAAM	Contractile Force After LAAM
1.4 mg/kg	None	82.8±3.8[b,c]	60.2± 6.1[b,c]	87.6± 3.1[b,c]
	Naltrexone[a]	95.3±5.1	82.6±17.1	87.5± 7.3
2.7 mg/kg	None	80.3±8.1[c]	50.6± 2.4[c]	67.0± 4.8[c]
	Naltrexone[a]	88.9±7.4	75.4±11.4[c]	87.2±18.2
5.5 mg/kg	None	74.1±1.7[c]	39.7± 1.1[c]	68.1± 8.4[c]
	Naltrexone[a]	87.6±6.7[c]	73.8± 2.0[c]	73.2±10.0[c]

[a] 5 μg/kg s.c. 35 minutes prior to LAAM injection.

[b] % of control, $\bar{X}$ ± S.E.M., N=3. Control values for all animals were: mean blood pressure, 125.3±4.5; heart rate, 154.5±4.4; contractile force, 70.0±3.2.

[c] Significantly less than pre-LAAM control, $p<.05$.

Figure 13. Effects of atropine pretreatment on mean blood pressure and heart rate responses to cumulative doses on LAAM in the cat. Bars and lines represent the $\bar{X}$±S.E.M., N=5. Asterisk indicates significantly different from pre-LAAM control, $p<.05$. Control values were: mean blood pressure (mmHg) 113.6±12.1; heart rate (beats/min) 190.0±12.0.

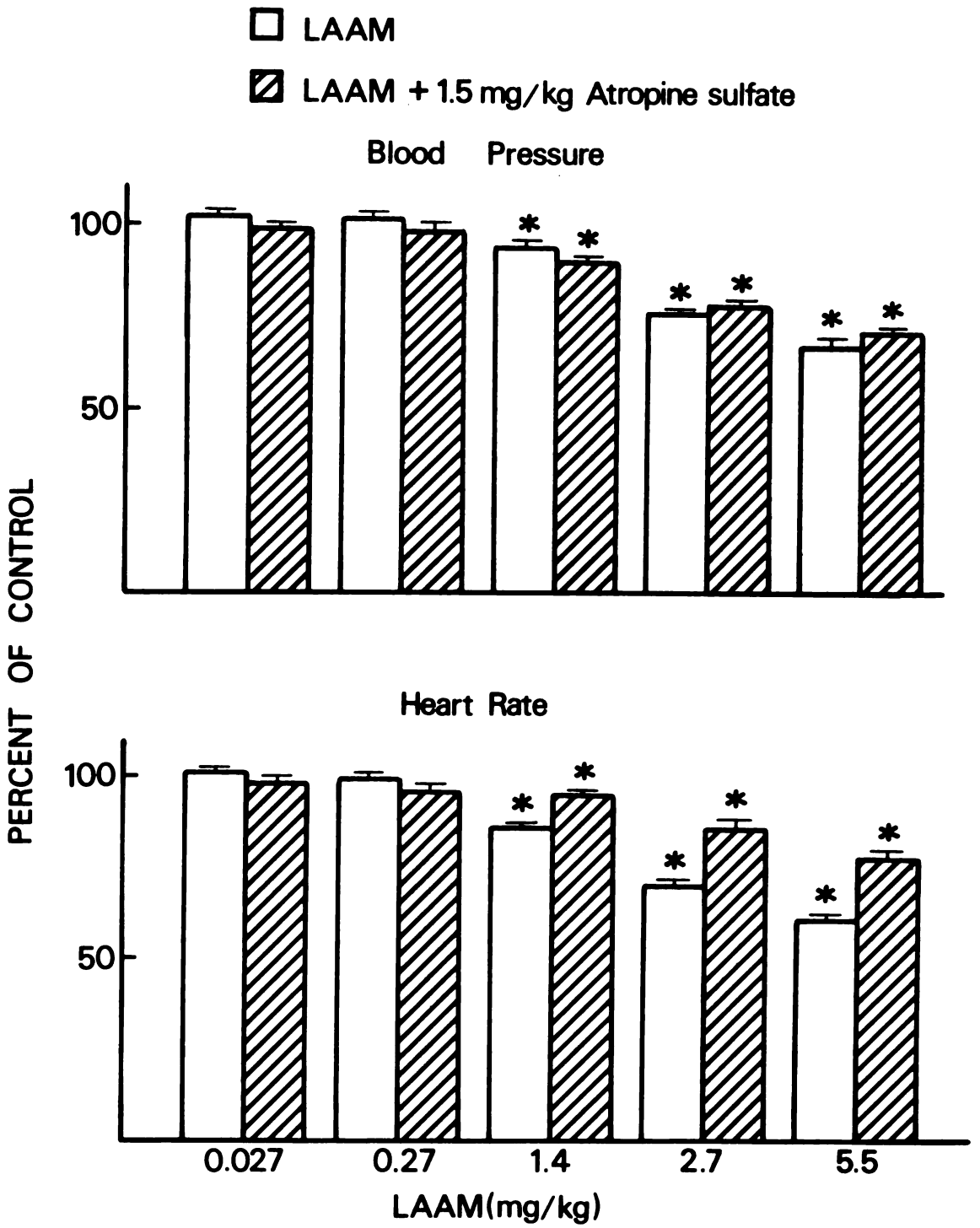
LAAM
LAAM + 1.5 mg/kg Atropine sulfate
Blood Pressure
100
50
Heart Rate
100
50
PERCENT OF CONTROL
0.027
0.27
1.4
2.7
5.5
LAAM(mg/kg)

Figure 13

cumulative doses of LAAM on BP and HR in cats where the responses of the nictitating membranes to pre- and postganglionic sympathetic nerves stimulation were monitored. The BP and HR responses to LAAM were similar to those previously discussed (Table 6). Atropine pretreatment had no significant effect on the BP response to LAAM. In contrast to this, atropine pretreatment significantly attenuated, but did not completely block, the effect of LAAM on HR. LAAM (5.5 mg/kg) decreased HR by 40.4±1.5% in control and by 23.6±1.2% in atropine pretreated animals. This is very similar to what had been observed previously in dogs (Stickney and Schwartz, 1978).

Frequency-response curves of the nictitating membranes to pre- and postganglionic sympathetic nerve stimulation before and after 2.7 mg/kg LAAM in a representative cat are shown in Figure 14. In this animal, LAAM decreased the nictitating membrane responses to pre- and postganglionic stimulation at low frequency (0.5-5.0 Hz) but not high frequency (15-20 Hz) stimulation. You will recall that BP and HR also are decreased significantly by this dose (2.7 mg/kg) of LAAM (Figure 13).

Figure 15 shows the effects of cumulative doses of LAAM on nictitating membrane responses to pre- and postganglionic sympathetic nerve stimulation at 1 Hz. At doses of 1.4 mg/kg LAAM or greater, the responses to both pre- and postganglionic stimulation were reduced significantly in a dose dependent manner. No significant differences between the effects of LAAM on pre- vs. postganglionic responses were observed. Atropine pretreatment did not affect the response to LAAM. These data indicate that similarities between the effects of LAAM on BP and its effects on the nictitating membrane responses to 1 Hz stimulation

Figure 14. Effects of LAAM on the frequency-response curves of the nictitating membranes to pre- and postganglionic sympathetic nerve stimulation. The upper and lower panels represent the nictitating membrane responses before and after 2.7 mg/kg LAAM. The numbers at the top of each panel are the various stimulation frequencies used in Hz. In this animal, LAAM decreases both preganglionic and postganglionic stimulation responses at the low frequencies (0.5, 1, 3, 5 Hz) but did not appear to affect the high frequency rsponses (15, 20 Hz).

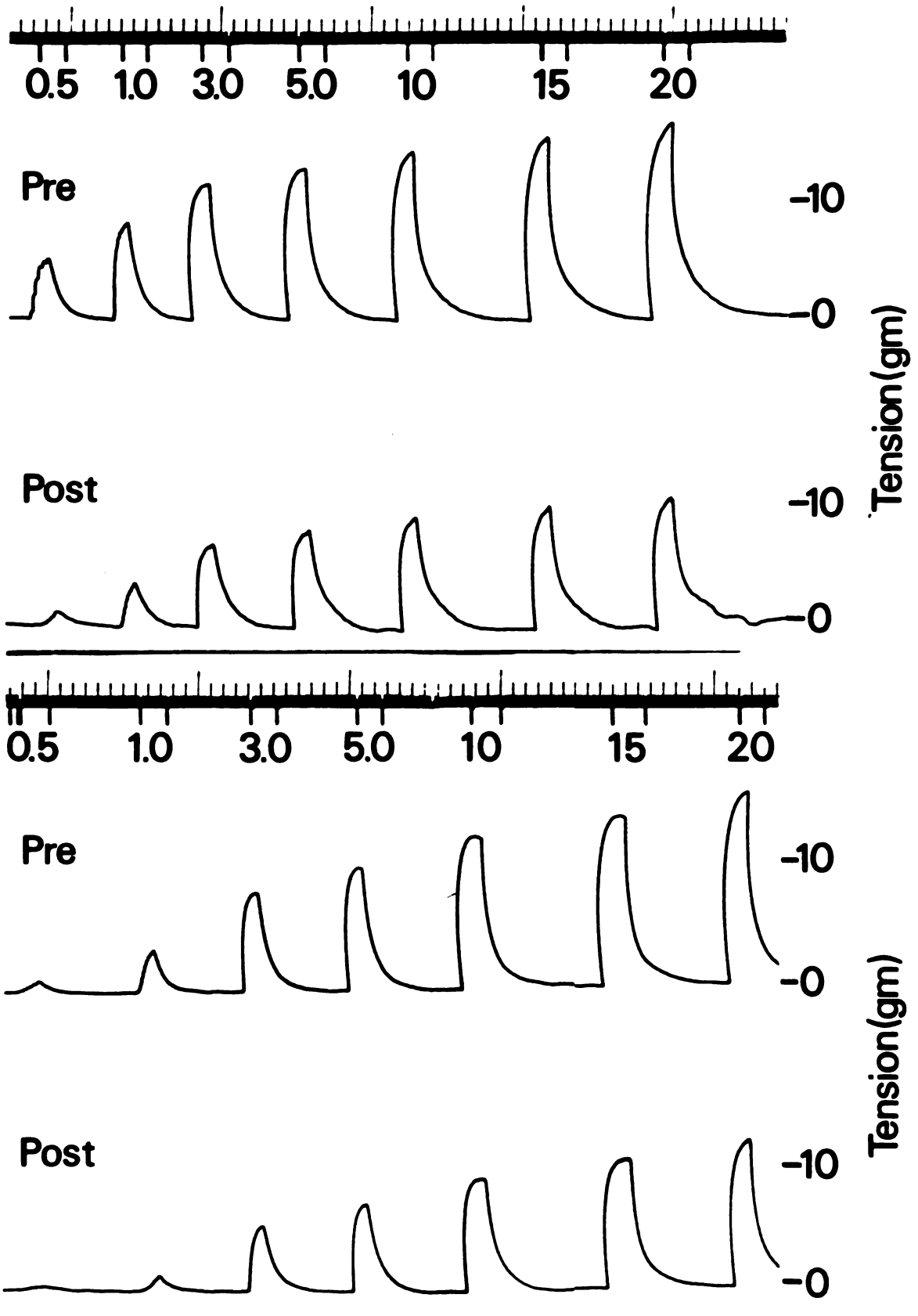
0.5
1.0
3.0
5.0
10
15
20
Pre
-10
-0
Post
-10
-0
Tension(gm)
0.5
1.0
3.0
5.0
10
15
20
Pre
-10
-0
Post
-10
-0
Tension(gm)

Figure 14

Figure 15. Effects of cumulative doses of LAAM on the responses of the nictitating membrane to pre- and postganglionic sympathetic nerve stimulation at 1 Hz. The bars and lines represent the nictitating membrane responses to stimulation expressed as a percent of the control response, $\bar{X}\pm$S.E.M., N=5. Asterisk indicates significant difference from pre-LAAM control, $p<.05$.

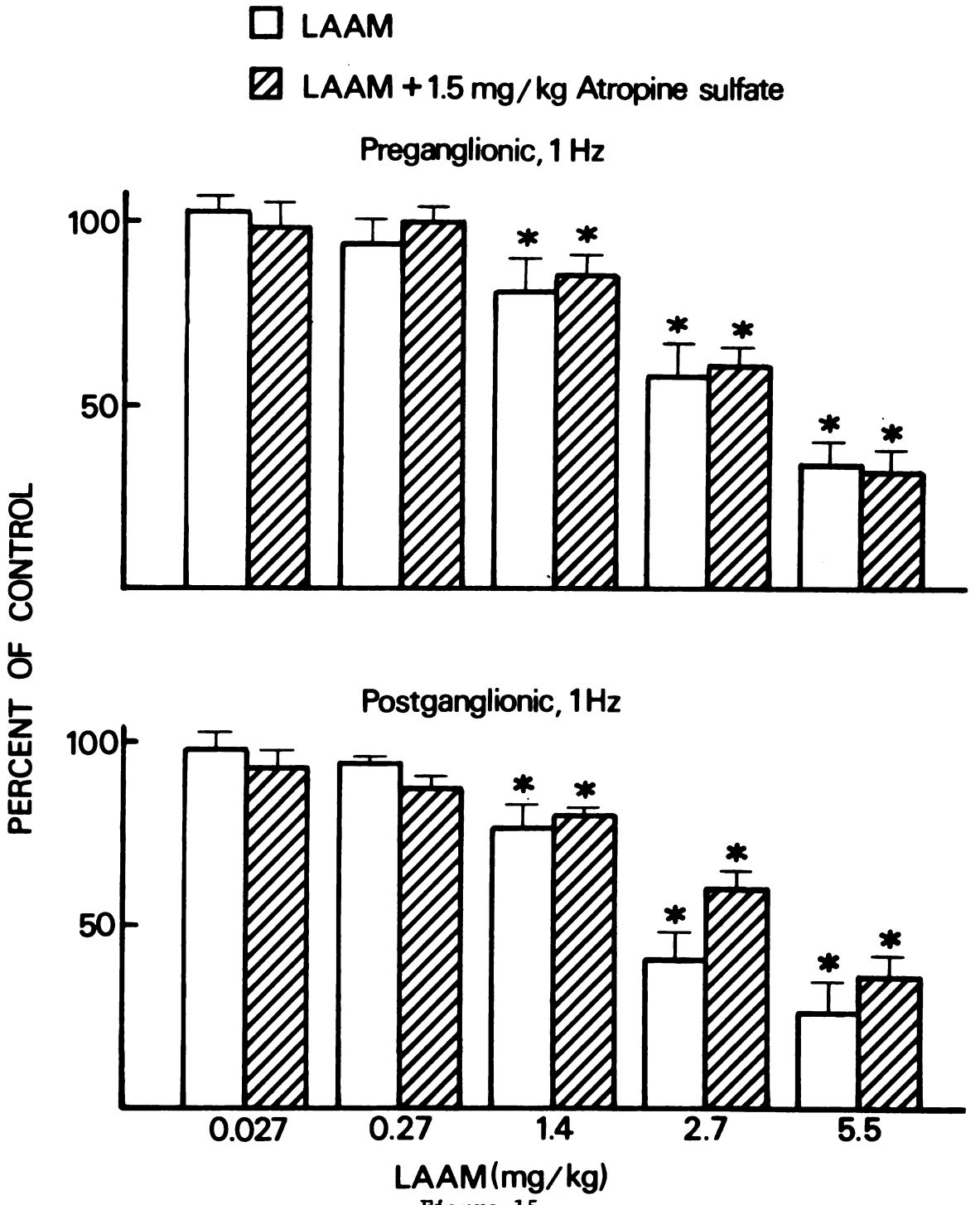
LAAM
LAAM + 1.5 mg/kg Atropine sulfate
Preganglionic, 1 Hz
Postganglionic, 1Hz
PERCENT OF CONTROL
100
50
0.027
0.27
1.4
2.7
5.5
LAAM(mg/kg)

Figure 15

exist. First, both effects have the same threshold dose, 1.4 mg/kg LAAM. Secondly, neither effect is antagonized by atropine pretreatment.

Figure 16 shows the effects of cumulative doses of LAAM on the nictitating membrane responses to pre- and postganglionic sympathetic nerve stimulation at 5 Hz. LAAM, at a dose of 2.7 mg/kg or higher, significantly decreased these responses. When compared to the effects of LAAM on the 1 Hz responses (Figure 15), several observations can be made. First, LAAM appears to have a greater effect on the 1 Hz responses. 5.5 mg/kg LAAM decreased 1 Hz pre- and postganglionic responses to approximately 27 and 33% of control, respectively, as compared to 70 and 55% of control, respectively, for the 5 Hz pre- and postganglionic responses. Neither the 1 Hz nor the 5 Hz responses showed a significant difference in the effects of LAAM on the pre- as opposed to postganglionic stimulation responses. Finally, the effects of LAAM, neither at 1 Hz nor at 5 Hz, were significantly altered by atropine pretreatment.

Figure 17 shows the effects of cumulative doses of LAAM on the responses of the nictitating membranes to pre- and postganglionic sympathetic nerve stimulation at 15 Hz. With the exception of the postganglionic response after 2.7 mg/kg LAAM only, nictitating membrane responses to sympathetic nerve stimulation in control and atropine pretreated animals were significantly decreased only after the highest dose of LAAM, 5.5 mg/kg. No significant differences between the effect of LAAM on pre- vs. postganglionic responses were found. The effects of 5.5 mg/kg LAAM on the 15 Hz responses were less than those observed

Figure 16. Effects of cumulative doses of LAAM on the responses of the nictitating membrane to pre- and postganglionic sympathetic nerve stimulation at 5 Hz. The bars and lines represent the nictitating membrane responses to stimulation expressed as a percent of the control response, X±S.E.M., N=5. Asterisk indicates significant difference from pre-LAAM control, $p<.05$.

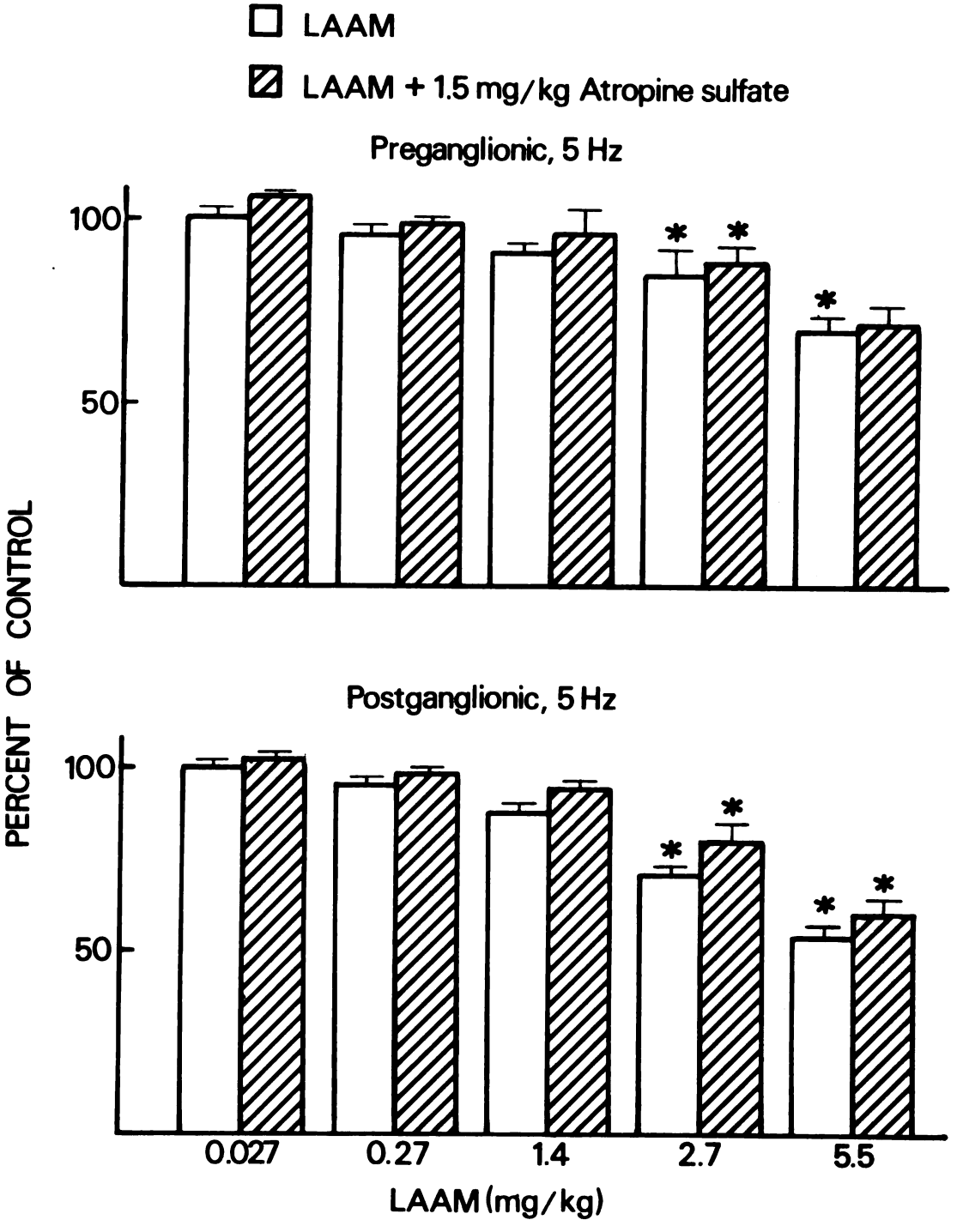
LAAM
LAAM + 1.5 mg/kg Atropine sulfate
Preganglionic, 5 Hz
100
50
Postganglionic, 5 Hz
100
50
PERCENT OF CONTROL
0.027
0.27
1.4
2.7
5.5
LAAM (mg/kg)

Figure 16

Figure 17. Effects of cumulative doses of LAAM on the responses of the nictitating membrane to pre- and postganglionic sympathetic nerve stimulation at 15 Hz. The bars and lines represent the nictitating membrane responses to stimulation expressed as a percent of the control response, $\bar{X}\pm$S.E.M., N=5. Asterisk indicates significant differenct from pre-LAAM control, $p<.05$.

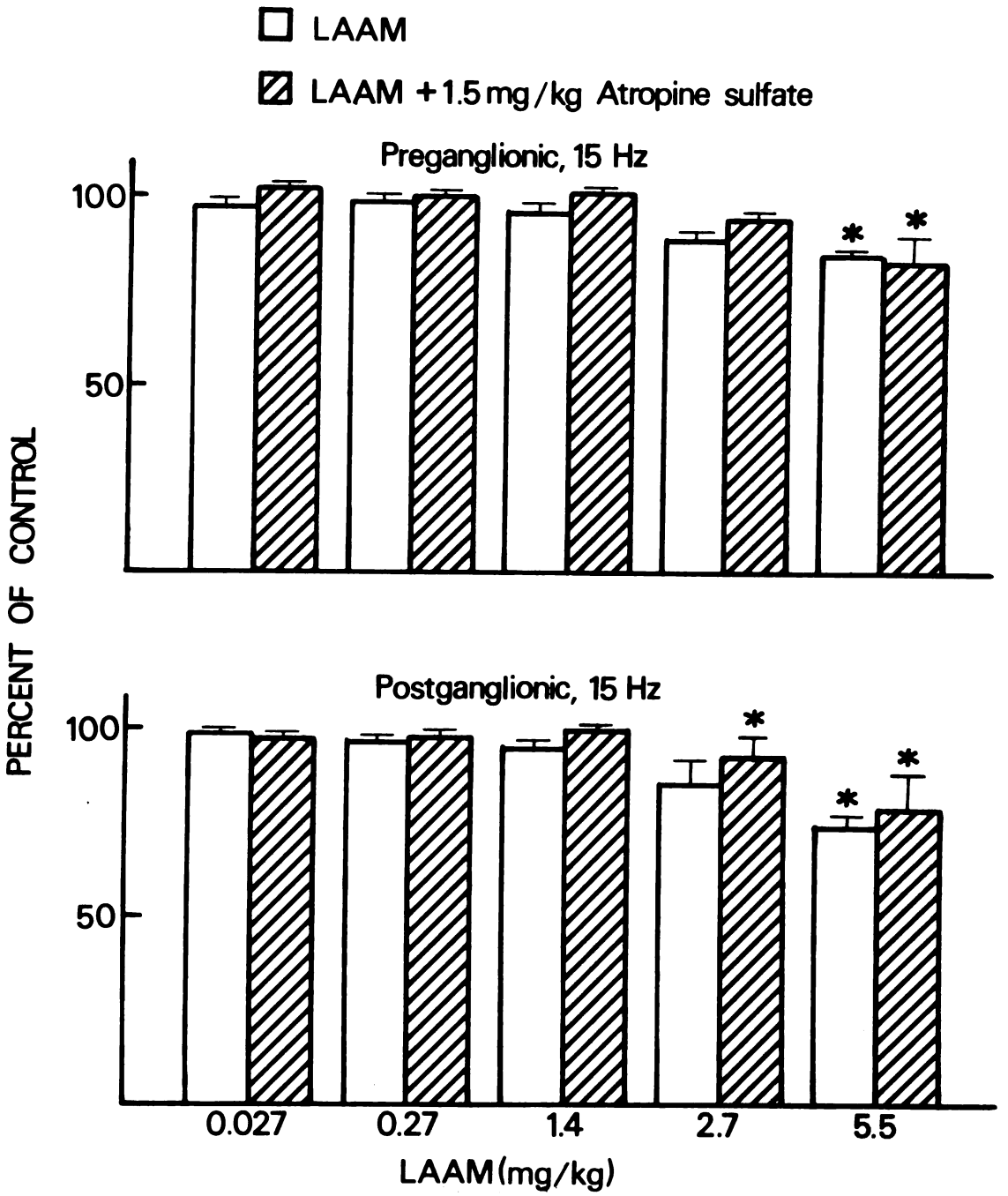
LAAM
LAAM +1.5 mg/kg Atropine sulfate
Preganglionic, 15 Hz
Postganglionic, 15 Hz
PERCENT OF CONTROL
100
50
0.027
0.27
1.4
2.7
5.5
LAAM(mg/kg)

Figure 17

for the 5 Hz (Figure 16) and 1 Hz (Figure 15) responses. In summary, the effects of LAAM on the nictitating membrane responses to pre- and postganglionic sympathetic nerve stimulation appear to be both dose and frequency dependent. The greatest effects are observed after the highest doses of LAAM and the responses to low frequency stimulation are more susceptible to effects of LAAM than are the high frequency responses. No differences exist between the effects of LAAM on pre- as opposed to postganglionic stimulation responses, suggesting that LAAM is not acting at the sympathetic ganglion. Finally, the minimum doses required for the effects of LAAM on the nictitating membrane responses to 1 Hz stimulation are the same as the minimum doses required for the BP and HR effects (Figure 13), suggesting the possibility of a common mechanism for these effects of LAAM.

The responses of the nictitating membranes to intravenous epinephrine, 5 μg/kg, also were observed in these animals (Table 12). At no dose in either control or atropine pretreated animals did LAAM significantly affect the nictitating membrane responses to epinephrine. These data indicate that a decrease in the responsiveness of the nictitating membrane to neurotransmitter is probably not the cause of the decreased responses of the nictitating membrane to nerve stimulation caused by LAAM.

In another group of animals, the effects of injections of LAAM, 2.7 mg/kg, and morphine, 13 mg/kg were examined. These studies were done to compare the effects of LAAM to those of an equieffective dose of morphine. 13 mg/kg morphine were required to produce cardiovascular effects similar to those of 2.7 mg/kg LAAM. The time course

TABLE 12

Effects of Cumulative Doses of LAAM on the Nictitating Membrane Responses to Intravenous Epinephrine (EPI), 5 µg/kg

Membrane	Pretreatment	LAAM Dose (mg/kg) 0.014	0.14	1.4	2.70	5.5
Right Side	None	114.6[a] ± 6.9	125.0 ±17.3	130.0 ±20.7	130.7 ±26.4	136.6 ±32.3
	Atropine 1.5 mg/kg	100.0 ±13.3	121.8 ±16.8	135.2 ±16.8	127.4 ±19.5	126.8 ±20.6
Left Side	None	108.4 ± 5.1	118.4 ±15.2	115.6 ±22.4	134.2 ±16.7	139.0 ±20.8
	Atropine 1.5 mg/kg	127.4 ±19.5	125.0 ±33.5	128.4 ±30.0	131.0 ±17.3	116.4 ±18.2

[a]Percent of control, $\bar{X}$ ± S.E.M.

for the effects of these treatments on BP, HR and the responses of the nictitating membranes to pre- and postganglionic sympathetic nerve stimulation are compared in Table 13. Both LAAM and morphine decreased BP to 71.0±10.6 and 61.4±5.9% of control, respectively, by 20 minutes after injection. By 240 minutes after injection, the BP effect of morphine had recovered significantly whereas no significant recovery was observed for LAAM. Both LAAM and morphine decreased HR to 72.0±5.7 and 69.9±5.0% of control, respectively, 20 minutes after injection. HR had returned to control 140 minutes after the morphine injection. No recovery was observed for LAAM even 230 minutes after injection. Both LAAM and morphine decreased the responses of the nictitating membranes to sympathetic nerve stimulation. Both had greater effects on the low frequency (1 Hz) responses and showed no significant differences in effects on pre- as opposed to postganglionic stimulation responses. No recovery was observed at 240 min after LAAM for these effects. However, by 240 min after morphine, significant recovery from its effects on the 1 Hz nictitating membrane responses was observed. These data would suggest that LAAM and morphine at the doses used produce similar effects but LAAM has a greater duration of action.

The effects of morphine (13 mg/kg) and LAAM (2.7 mg/kg) on BP, HR and the nictitating membrane reponses to pre- and postganglionic sympathetic nerve stimulation in naltrexone pretreated (3 mg/kg) animals were studied. Earlier experiments had shown that naltrexone could not completely antagonize the HR response to LAAM. The present experiment was performed to compare the effect of naltrexone pretreatment on the responses to equieffective doses of morphine and LAAM.

TABLE 13

Comparison of the Effects of LAAM (L)[a] and Morphine (M)[b] on Blood Pressure, Heart Rate and the Nictitating Membrane Responses to Nerve Stimulation in the Cat

Time After Injection (min)	Mean Blood Pressure L	Mean Blood Pressure M	Heart Rate L	Heart Rate M	Nictitating Membrane Stimulation Responses Preganglionic 1.0 Hz L	Preganglionic 1.0 Hz M	Preganglionic 10.0 Hz L	Preganglionic 10.0 Hz M	Postganglionic 1.0 Hz L	Postganglionic 1.0 Hz M	Postganglionic 10.0 Hz L	Postganglionic 10.0 Hz M
20	71.0[c,d] ±10.6	61.4 ±5.9	72.0 ±5.7	69.9 ±5.0	50.3 ±6.9	36.8 ±2.2	92.7 ±0.9	86.6 ±2.0	41.1 ±4.5	15.9 ±8.6	87.4 ±1.6	71.3 ±6.4
50	72.7 ±6.4	68.6 ±2.3	67.0 ±8.7	78.6 ±5.9	28.5 ±9.5	27.2 ±4.4	84.7 ±2.9	81.8 ±2.0	24.4 ±6.7	14.6 ±4.1	84.9 ±6.8	63.8 ±7.1
100	72.7 ±5.6	78.9 ±5.5	65.7 ±4.1	90.2[e] ±12.4	17.2 ±8.5	42.7 ±9.4	87.9 ±2.7	86.5 ±5.1	16.8 ±6.7	20.9 ±6.0	78.0 ±5.1	69.9 ±5.9
230	79.3 ±4.8	83.9[f] ±9.1	75.0 ±2.9	101.2[e,f] ±10.6	22.3 ±5.8	47.1[f] ±7.3	74.7 ±8.1	84.7 ±4.7	16.2 ±6.9	33.6[f] ±7.5	73.1 ±7.2	72.1 ±1.8

[a]LAAM, 2.7 mg/kg. [b]Morphine sulfate, 13.3 mg/kg. [c]Percent control ($\bar{X}$ ± S.E.M.). [d]All values significantly less than control except where indicated otherwise. [e]Significantly less than control, $p<.05$. [f]Significantly different from 50 min values. For control values of mean blood pressure and heart rate, see Table 14.

Figure 18 shows the data for morphine. Naltrexone completely antagonized the effects of morphine on BP, HR and the nictitating membrane responses to sympathetic nerve stimulation. Figure 19 shows the data for LAAM. Naltrexone completely antagonized the effects of LAAM on BP and on the responses of the nictitating membranes to sympathetic nerve stimulation. Naltrexone partially antagonized, but did not completely block, the effect of LAAM on HR. These data suggest that the primary mechanisms for the effects of LAAM and morphine on BP and on the responses of the nictitating membranes to sympathetic nerve stimulation involve an interaction of these drugs with opiate binding sites. However, only the effect of morphine on HR was completely antagonized by naltrexone. This suggests that the primary mechanism for the effect of morphine on HR involves an interaction of morphine with opiate binding sites. Other mechanisms in addition to an interaction with opiate binding sites appear to contribute significantly to the HR response to LAAM. In general, the effects of naltrexone on the BP and HR effects of LAAM in the cat are similar to what was observed in the dog (Table 8).

In a final experiment, an attempt was made to overcome the antagonism of naltrexone on the BP effect of LAAM. The data, shown in Table 14, indicate that even at doses 3 times greater than those involved in Figure 19, 8.20 mg/kg, the effect of LAAM on BP was completely blocked, as were its effects on the nictitating membrane responses (data not shown). However, a dose dependent increase in the effect of LAAM on HR was observed, even in the presence of naltrexone. These data further support the suggestion that a portion of the

Figure 18. Effect of morphine, 13 mg/kg, i.v., on mean arterial blood pressure, heart rate, and the nictitating membrane responses to pre- and postganglionic sympathetic nerve stimulation. Nictitating membrane responses to epinephrine (EPI), 5 μg/kg i.v., also were monitored. The lower two panels represent the nictitating membrane responses expressed as a percent of the control responses. Bars and lines represent the $\bar{X}\pm$S.E.M., N=3. Asterisk indicates significantly different from pre-morphine control, $p<.05$.

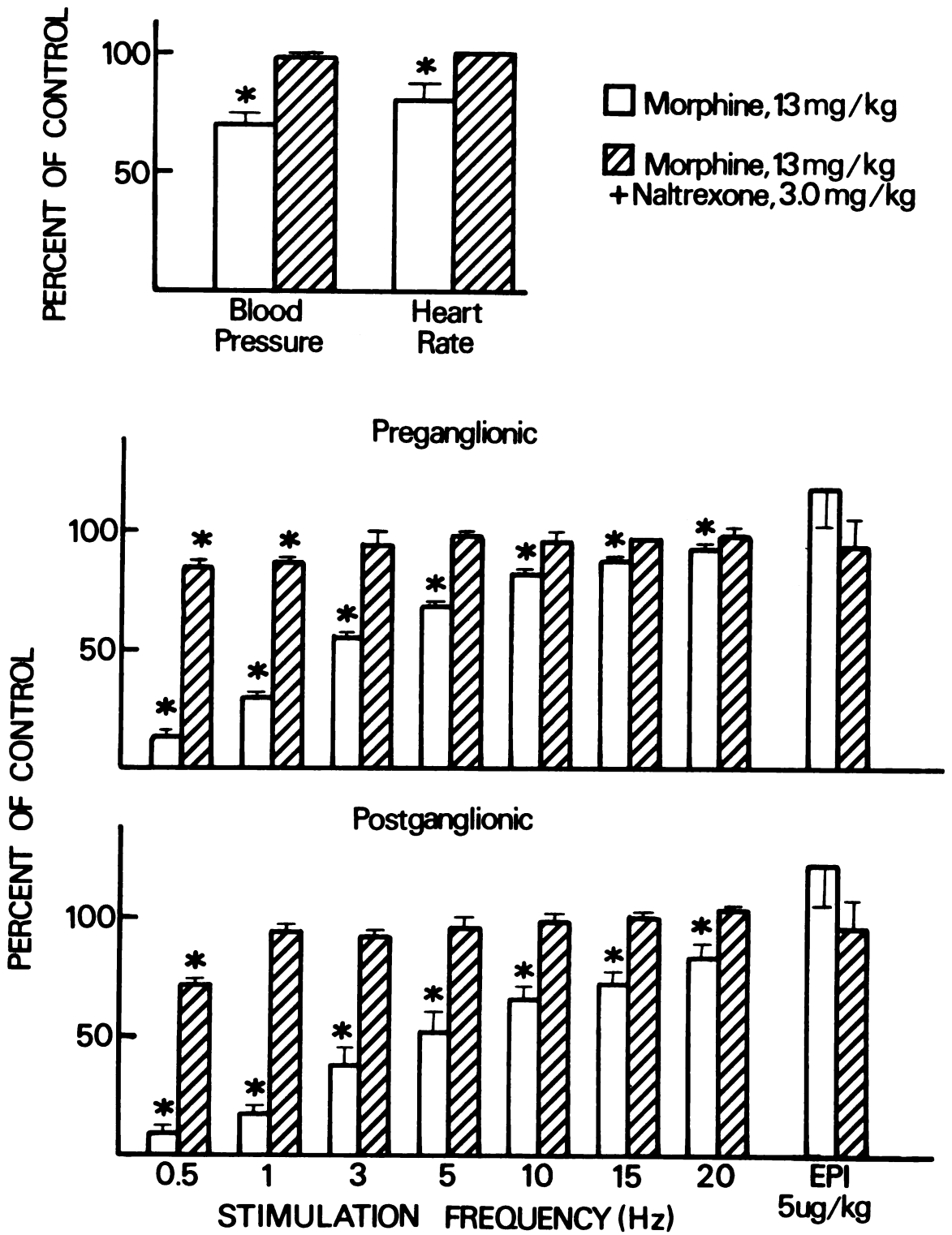
PERCENT OF CONTROL
100
50
Blood Pressure
Heart Rate
Morphine, 13 mg/kg
Morphine, 13 mg/kg + Naltrexone, 3.0 mg/kg
Preganglionic
Postganglionic
0.5
1
3
5
10
15
20
EPI 5ug/kg
STIMULATION FREQUENCY (Hz)

Figure 18

Figure 19. Effects of LAAM, 2.7 mg/kg i.v., on mean arterial blood pressure, heart rate and the nictitating membrane responses to pre- and postganglionic sympathetic nerve stimulation. Nictitating membrane responses to epinephrine (EPI), 5 μg/kg i.v., also were monitored. The lower two panels represent the nictitating membrane responses expressed as a percent of the control responses. Bars and lines represent the $\bar{X}\pm$S.E.M., N=3. Asterisk indicates significantly different from pre-LAAM control, $p<.05$.

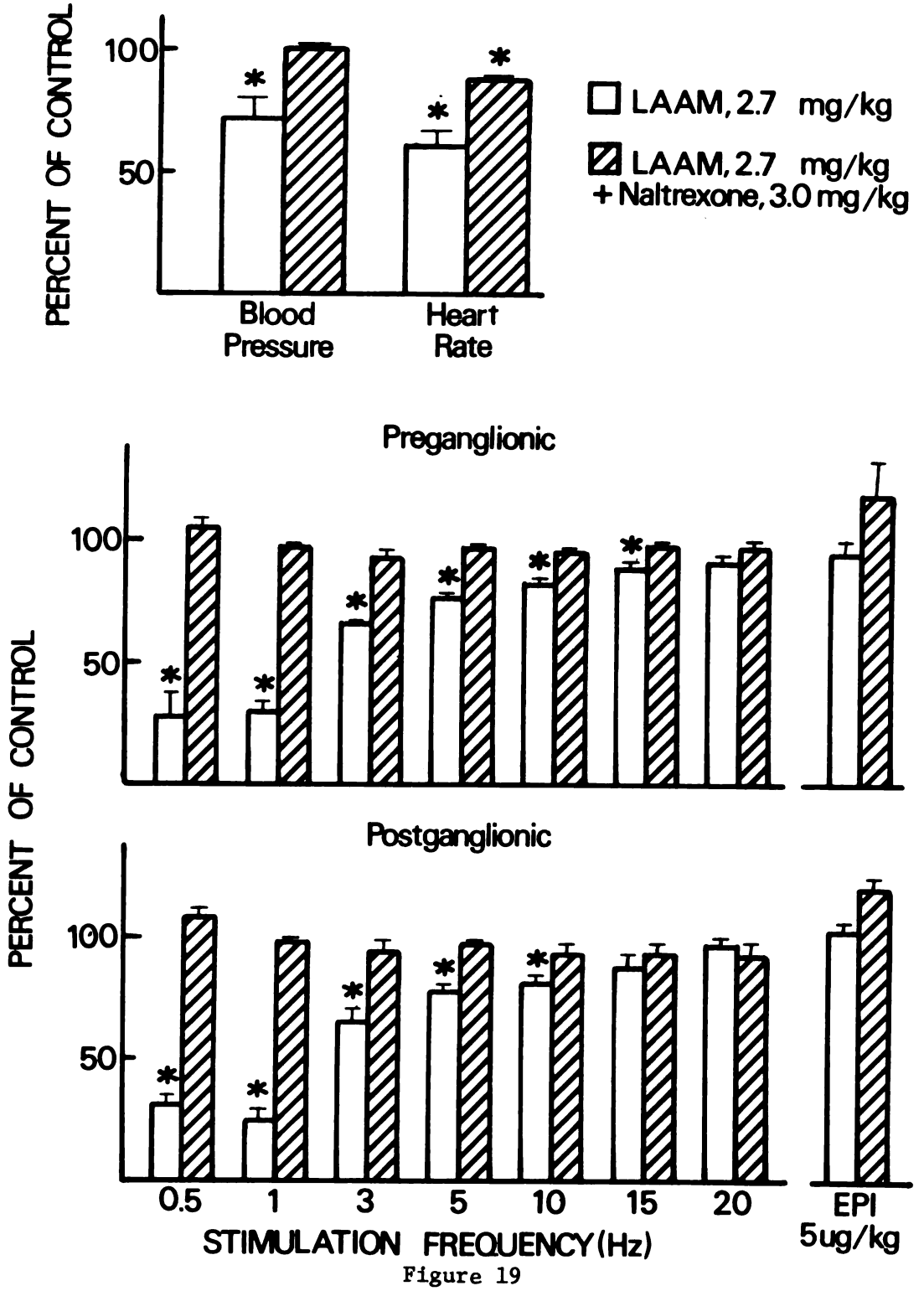
PERCENT OF CONTROL
100
50
Blood Pressure
Heart Rate
LAAM, 2.7 mg/kg
LAAM, 2.7 mg/kg + Naltrexone, 3.0 mg/kg
Preganglionic
Postganglionic
0.5
1
3
5
10
15
20
EPI 5ug/kg
STIMULATION FREQUENCY (Hz)

Figure 19

TABLE 14

Effects of Naltrexone (3 mg/kg) Pretreatment on Blood Pressure and Heart Rate Responses to LAAM in the Cat

Dose of LAAM (mg/kg)	Pretreatment[d]	Mean Blood Pressure[a]	Heart Rate[a]
2.7	None	72.7±6.4[b]	67.0±8.7[b]
	Naltrexone	104.5±4.5	89.1±4.9[b]
5.5	None	62.3±3.8[b]	64.3±1.5[b]
	Naltrexone	98.7±1.3	83.3±2.0[b]
8.2	None	54.7[c]	50.7[c]
	Naltrexone	98.3[c]	74.3[c]

[a] % of control ($\bar{X}$ ± S.E.M.), N=3. Control values were: mean blood pressure, 107.4 8.5 ; heart±rate, 192.6±9.4.

[b] Significantly less than pre-LAAM control, $p<.05$.

[c] $\bar{X}$, N=2.

[d] 3 mg/kg, s.c.

bradycardia caused by LAAM *in vivo* does not require the interaction of LAAM with opiate binding sites.

In summary, the effects of LAAM on the nictitating membrane responses to sympathetic nerve stimulation have much in common with the cardiovascular effects of LAAM. The minimum doses required to produce the effects of LAAM on BP, HR and the nictitating membrane responses are the same, 1.4 mg/kg. Furthermore, the effects of LAAM on BP, HR and the nictitating membrane responses are antagonized by naltrexone. Lastly, the timecourses for the effects of LAAM on the nictitating membrane responses, BP, and HR are very similar. These data suggest that effects of LAAM on the peripheral sympathetic nervous system may be causally related to the cardiovascular actions of LAAM.

DISCUSSION

The objective of the present study was to examine, and identify mechanisms for the in vivo cardiovascular actions of LAAM and its two major metabolites nor-LAAM and dinor-LAAM. The similarities and differences between the effects of LAAM and its metabolites were examined first. Mean arterial blood pressure (BP), heart rate (HR), contractile force (CF) and Lead II electrocardiogram (ECG), as well as the responses to bilateral carotid artery occlusion (BLCO) were measured. Additional experiments evaluated the relative contribution of cardiac and vascular changes to the effects of LAAM on BP. The second set of experiments examined the involvement of the autonomic nervous system in the mechanisms for the cardiovascular actions of LAAM. The involvement of opiate binding sites in the mechanisms for the cardiovascular actions of LAAM was evaluated in the third set of experiments. The final set of experiments was designed to determine the effects of LAAM on the peripheral sympathetic nervous system and the possible contribution that these effects make to the cardiovascular actions of LAAM.

A. Comparison of the In Vivo Effects of LAAM, nor-LAAM, and dinor-LAAM on the Cardiovascular System

Comparison of the in vivo cardiovascular effects of LAAM and its metabolites is both relavent and necessary for several reasons. First

nor-LAAM and dinor-LAAM are pharmacologically active opiates. Both compounds have analgesic actions which are antagonized by naloxone and both inhibit electrically-induced contraction of the guinea pig ileum (Chen, 1948; Veatch et al., 1964; Nickander et al., 1974). Another reason is that LAAM and its metabolites produce negative inotropic and chronotropic effects in vitro. Finally, nor-LAAM and dinor-LAAM cumulate in patients chronically receiving LAAM, resulting in significant amounts of nor-LAAM and dinor-LAAM being present in the plasma of these patients (Kaiko and Inturrisi, 1975; Henderson et al., 1977a). Therefore, the effects of nor-LAAM and dinor-LAAM may be as important as the effects of LAAM in the clinical situation and warrant investigation.

Nor-LAAM and dinor-LAAM, as well as LAAM, decreased BP, HR, and CF in anesthetized dogs. Lower doses of nor-LAAM were required to decrease BP and HR than of LAAM or dinor-LAAM. Examination of the dose-response curves suggests that nor-LAAM is more potent than LAAM or dinor-LAAM in producing these effects. In contrast to this, the lowest doses of LAAM, nor-LAAM and dinor-LAAM which significantly decreased CF were the same and the 3 compounds appeared to be equipotent. The greater potency of nor-LAAM in reducing BP and HR emphasizes the need to consider the actions of the metabolites of LAAM.

The cardiovascular response to LAAM is not unique to the dog; LAAM also decreases BP, HR, and CF in the anesthetized cat. The minimum dose of LAAM required to decrease BP, HR, and CF was 1.4 mg/kg, identical to the minimum dose required in the dog.

The increases in BP, HR, and CF observed in response to BLCO were significantly decreased by LAAM in anesthetized dogs. This effect was observed at the same doses which decreased BP, HR, and CF. LAAM also decreased cardiac output. After 5.5 mg/kg LAAM, BP and cardiac output were reduced by 35% and 52%, respectively. The reduction of BP and cardiac output, in combination with the impairment of the BLCO responses, suggests that LAAM may alter the function of homeostatic mechanisms which normally maintain BP.

It was decided that the effects of LAAM on cardiac output and BP should be compared with those of an agent whose mechanism of action was specific to the heart. The agent chosen was practolol, a selective β_1-adrenergic blocking agent. Dunlop and Shanks (1968) demonstrated that practolol produces cardioselective β-adrenergic blockade at doses up to 5 mg/kg. The rationale behind comparing the effects of LAAM and practolol was that any changes in BP caused by practolol would have to be the result of its effects on cardiac output. Thus, differences between the BP effects of LAAM and practolol, when cardiac output decreases were similar, could be attributed to effects of LAAM at sites other than the heart. LAAM produced larger decreases in blood pressure than practolol when decreases in cardiac output were similar. Calculated total peripheral resistance (TPR) increased following administration of either drug. The increase in TPR after LAAM appeared to be less than the increase in TPR after practolol when cardiac output decreases were comparable. While the TPR data are not conclusive, the fact that LAAM caused much larger decreases in BP than practolol when decreases in cardiac output were comparable would suggest that LAAM impaired the function of homeostatic mechanisms which attempt to maintain

BP when cardiac output is decreased. This impairment could be the result of a central action by LAAM to alter baroreceptor reflex function, a peripheral action to decrease reflex activation of the vasculature by decreasing norepinephrine release from sympathetic nerve terminals or a direct vascular action to decrease the response of the vascular smooth muscle to neurotransmitter and/or act as a vasodilator.

The anesthetized state of the animals should be considered when the results of the present study are evaluated. Sodium pentobarbital acts centrally to decrease vagal activity and thereby causes an increase in heart rate above that normally observed in resting conscious animals (Nash et al., 1956; Olmstead and Page, 1966). This effect has been shown to influence cardiovascular responses to certain drugs. For example, Δ^9-THC produces only a slight decrease in heart rate in conscious dogs but causes a substantial bradycardia in animals anesthetized with sodium pentobarbital (Jandhyala and Buckley, 1977). Are the negative chronotropic responses to LAAM, nor-LAAM, and dinor-LAAM that were observed the consequence of such a drug-drug interaction? Apparently, they are not. Recently, LAAM has been reported to significantly decrease heart rate and cardiac output in conscious dogs (Waters et al., 1978). In fact, on a percent of control basis, slightly smaller doses of LAAM produced a greater bradycardia in conscious animals than that observed in anesthetized animals. Bradycardia also has been reported in monkeys chronically receiving LAAM (Masten et al., 1978).

Species differences represent another factor to be considered when evaluating the present findings. The dog and cat may be uniquely

sensitive to the cardiovascular effects of LAAM and its metabolites and the data may be irrelevant to man. LAAM, nor-LAAM, and dinor-LAAM produce both inotropic and chronotropic responses in isolated cardiac tissues from rats, guinea pigs, and rabbits as well (Stickney and Keedy, 1978). It is unlikely that man is insensitive to the cardiovascular actions of LAAM.

The *in vivo* cardiovascular actions of LAAM and its metabolites should also be considered in relation to the cardiovascular actions of other narcotics. *In vitro* and *in vivo* studies have indicated that the cardiovascular actions of all narcotic analgesics are not similar to those of morphine (see Introduction). Agents such as morphine, fentanyl and dextromoramide, hereafter referred to as group I agents, appear to have similar actions on the heart at equianalgesic doses (Strauer, 1972, 1974; Laubie *et al.*, 1974; Daskalopoulos *et al.*, 1975; Jaffe and Martin, 1975). In contrast to this, other agents including methadone, meperidine, propoxyphene, and nor-propoxyphene, hereafter referred to as group II agents, appear to be more potent cardiodepressants than equianalgesic doses of the group I compounds (Scott and Chen, 1946; Sugioka *et al.*, 1957; Strauer, 1972, 1974; Jaffe and Martin, 1975; Stickney, 1977a,b; Holland and Steinberg, 1979). *In vitro* studies suggest that LAAM and its metabolites are group II agents. Although LAAM and morphine have similar analgesic potencies (Eddy *et al.*, 1950), LAAM produced significant cardiodepressant effects of concentrations at which morphine had no effect (Stickney, 1977a,b). This might be anticipated since LAAM is a derivative of methadone. The results of the present study suggest that LAAM and its metabolites act

as group II compounds *in vivo* as well. As little as 1.4 mg/kg LAAM or 0.14 mg/kg nor-LAAM significantly decreased HR and CF.

In summary, the findings presented herein demonstrate that LAAM, nor-LAAM and dinor-LAAM can significantly decrease BP, HR, and CF. Nor-LAAM appears to be more potent with regard to its hypotensive and negative chronotropic activity than LAAM or dinor-LAAM. However, although quantitative differences exist, the cardiovascular actions of LAAM and its metabolites appear to be qualitatively similar.

B. Mechanisms for the Cardiovascular Actions of LAAM

1. General Involvement of the Autonomic Nervous System in the Mechanisms for the Cardiovascular Actions of LAAM

The results considered in the previous section contain several lines of evidence pointing to the autonomic nervous system as a possible site of action for LAAM. Previous experiments have shown that the negative chronotropic effects of LAAM *in vivo* are antagonized by atropine, suggesting the involvement of the vagus in the mechanism of action (Stickney and Schwartz, 1978). Lastly, as mentioned in the Introduction, several narcotics have been shown to produce cardiovascular responses via actions on the autonomic nervous system (Laubie *et al.*, 1974). Therefore, the involvement of the autonomic nervous system in the cardiovascular actions of LAAM was examined.

a. The parasympathetic nervous system

Bilateral cervical vagotomy (VAGOT) did not alter the cardiovascular response to LAAM significantly. Atropine does not antagonize the BP effect of LAAM but partially antagonizes the negative chronotropic actions of LAAM *in vivo* (Stickney, 1978a). These data

suggest that LAAM is acting through muscarinic cholinergic receptors but not by increasing vagal activity. Increased vagal activity is an important mechanism for the bradycardia produced by some narcotic analgesics (Kayaalp and Kaymakcalan, 1966; Fennessy and Rattray, 1969; Laubie et al., 1974). As discussed in the previous section, there appear to be 2 groups of narcotics which differ with regard to their relative potency as cardiodepressants. Experiments in vagotomized animals suggest that increased vagal activity is the primary mechanism for the bradycardia produced by group I agents. These narcotics have minimal effects on HR in vagotomized animals (Laubie et al., 1974; Kayaalp and Kaymakcalan, 1966). Group II agents appear to be different. Sugioka and co-workers (1957) found that atropine attenuated, but did not completely block, the negative chronotropic activity of meperidine. Additional experiments led them to conclude that mechanisms in addition to increased vagal tone are important in meperidine-induced bradycardia. Propoxyphene and its metabolite nor-propoxyphene have been shown to have direct effects on the heart which appear to contribute to the negative chronotropic actions observed in vivo when the doses used do not exceed the threshold dose for central nervous system toxicity (Holland and Steinberg, 1979). Therefore, mechanisms other than, or in addition to an increase in vagal tone appear to be involved in the negative chronotropic actions of LAAM and other group II compounds.

Since atropine attenuated the bradycardia produced by LAAM in vivo, muscarinic cholinergic receptor activation appears to be involved

in LAAM's negative chronotropic action. Isolated right atrium studies have shown that the negative chronotropic effect of LAAM is comprised of two components (Stickney, 1978a). Atropine ($1x10^{-6}M$) completely blocks the effect of low concentrations of LAAM ($5x10^{-6}M$) but only attenuates the effects of higher concentrations. The same concentration of atropine shifts the dose-response curve for methacholine in isolated guinea pig right atria by more than 2 orders of magnitude (Stickney, 1978a). It would appear that, at lower concentrations, the negative chronotropic activity of LAAM is totally attributable to a mechanism which involves muscarinic receptors. The mechanisms involved in the *in vitro* bradycardia may help to explain the mechanisms for the negative chronotropic actions of LAAM *in vivo* which are blocked by atropine. Several general explanations can be proposed. LAAM may be acting directly as a muscarinic cholinergic agonist or perhaps it is acting indirectly by increasing the amount of endogenous acetylcholine binding to muscarinic cholinergic receptors. A combination of these two mechanisms is also possible.

No evidence is available proving or disproving the possibility that LAAM acts directly as a muscarinic agonist. However, the fact that LAAM can enhance the negative chronotropic activity of methacholine *in vitro* suggests that indirect mechanisms are also involved in the muscarinic actions of LAAM (Stickney, 1978a). There are 3 ways by which LAAM could act indirectly through muscarinic receptors. One of these mechanisms is by causing release of tissue acetylcholine.

This is unlikely since narcotics characteristically act to inhibit acetylcholine release (Paton, 1957; Schaumann, 1957; Cox and Weinstock, 1966; Lees et al., 1973). Inhibition of acetylcholine release is the mechanism by which opiates inhibit the electrically induced contraction of the guinea pig ileum (Schaumann, 1957). Another way LAAM could indirectly increase muscarinic receptor interactions is by cholinesterase inhibition. Several investigators have demonstrated inhibition of human serum and human erythrocyte cholinesterase by other narcotics such as codeine, levorphanol, meperidine and methadone (Young et al., 1955; Foldes et al., 1959; Ettinger and Gero, 1966). More recently, Gero (1978) has shown that LAAM competitively inhibits human serum esterase. LAAM enhanced the negative chronotropic activity of methacholine, a substrate for cholinesterase, in the same manner as physostigmine, a known cholinesterase inhibitor (Stickney, 1978a). Cholinesterase inhibition by LAAM could decrease acetylcholine inactivation in isolated atria which in turn could decrease the rate of beating. Anticholinesterase activity might also contribute to the effects of LAAM in vivo which are antagonized by atropine. A third possibility is that LAAM increases the affinity of muscarinic cholinergic receptors for their agonists. If LAAM acted allosterically to increase muscarinic receptor affinity, LAAM would increase the potency of endogenous as well as exogenous agonists. This would explain how LAAM could appear to act as a muscarinic cholinergic agonist and also act to increase the effects of other agonists. Although such a mechanism has not been identified for cholinergic receptors, substantial evidence now exists for such a mechanism at GABA receptors (Costa et al., 1975; Choi et al., 1977; MacDonald and Barker, 1978). Benzodiazepines appear to enhance GABA

mediated neurotransmission, enhance the effects of GABA agonists, and appear to act as direct GABA agonists. However, benzodiazepines do not bind to GABA receptors. Recently, it was shown that benzodiazepines bind at an allosteric site, displacing an inhibiting protein, thereby increasing the affinity of GABA receptors (Guidotti et al., 1978).

The effects of LAAM, nor-LAAM and dinor-LAAM on purified butyrylcholinesterase, purified acetylcholinesterase, guinea pig plasma and guinea pig heart homogenate cholinesterase activities were examined. LAAM and its metabolites were much more potent inhibitors of butyrylcholinesterase and plasma cholinesterase than of acetylcholinesterase and heart homogenate cholinesterase. The similarities between the effects of LAAM and its metabolites on butyrylcholinesterase and plasma cholinesterase may be related to the fact that guinea pig plasma cholinesterase is made up, primarily, of butyrylcholinesterase (Augustinson, 1948). Furthermore, the similarity between the effects of these drugs on acetylcholinesterase and heart homogenate cholinesterase suggests that acetylcholinesterase is the predominant enzyme in the heart homogenate.

LAAM and its metabolites were compared to other narcotics with regard to anticholinesterase activity. The effects of methadone on the heart homogenate and plasma enzymes were very similar to those of LAAM, nor-LAAM, and dinor-LAAM. In contrast to this, morphine at the same concentrations did not inhibit the activity of either enzyme. LAAM and methadone possess significant negative chronotropic activity whereas morphine does not (Stickney, 1977a). Taken together, these two sets of data might be interpreted as indirect evidence that

cholinesterase inhibitory activity plays a role in the actions of LAAM and methadone to decrease heart rate. Cholinesterase inhibition has been identified as the mechanism for effects produced by other narcotics. The phenomenon of acute tolerance to various narcotics in the guinea pig ileum and morphine-induced supersensitivity of the frog rectus muscle to acetylcholine both are the result of anticholinesterase actions by narcotics (Kosterlitz and Waterfield, 1975; Turlapaty et al., 1977).

LAAM, nor-LAAM and dinor-LAAM inhibited heart homogenate cholinesterase at a concentration of $1x10^{-4}$M. The magnitude of inhibition was 16-18%. This could result in a slowing of isolated right atria. Burn and Kottegoda (1953) showed changes in amplitude and rate and of isolated rabbit right atria in the presence of physostigmine. The concentration of physostigmine used produced not more than 16% inhibition of acetylcholine hydrolysis in auricle homogenates in vitro. An important point in considering the present data, however, is that the concentration at which cholinesterase inhibition is observed is $1x10^{-4}$M whereas the concentration of LAAM which produces decreases in heart rate totally attributable to a cholinergic mechanism is $5x10^{-6}$M (Stickney, 1978a). No cholinesterase inhibition was demonstrated at this concentration of LAAM (data not shown). This finding would suggest that at low concentrations ($5x10^{-6}$M) LAAM does not decrease heart rate by cholinesterase inhibition, since none was observed, but that cholinesterase inhibition may play a role at the higher concentrations where responses are attenuated but not blocked completely by atropine.

Realizing the limitations of comparing in vitro enzyme inhibition in homogenates to effects on isolated atria, additional

evidence was sought to support the hypothesis that low concentrations of LAAM do not cause bradycardia by inhibiting cholinesterase. This evidence is provided by the experiments wherein the effects of LAAM and physostigmine on the concentration-effect curves for bethanechol in isolated guinea pig right atria were studied. Bethanechol is a cholinergic agonist that is not a substrate for cholinesterase (Koelle, 1975). Therefore, a cholinesterase inhibitor should not enhance the actions of bethanechol, at least not within 1-2 hours. The known cholinesterase inhibitor, physostigmine, had no effect on the negative chronotropic response of isolated guinea pig right atria to bethanechol. In contrast to this, $5x10^{-6}$M LAAM significantly potentiated the negative chronotropic response to bethanechol. This finding argues against the involvement of cholinesterase inhibition in the negative chronotropic action of low LAAM concentrations ($5x10^{-6}$M) but does not eliminate the possibility that anticholinesterase activity contributes to the negative chronotropic responses observed following higher concentrations of LAAM.

In summary, the cardiovascular response to LAAM *in vivo* does not appear to involve changes in vagus nerve activity. However, muscarinic receptors do appear to play a role in the mechanism whereby LAAM decreases HR both *in vivo* and *in vitro*. Investigations into the anticholinesterase activity of LAAM suggest that only at high concentrations could cholinesterase inhibition contribute to the negative chronotropic action of LAAM. Furthermore, such high concentrations were probably not attained *in vivo* based on the calculations of plasma drug levels discussed earlier. It would appear that the negative chronotropic action of LAAM which involves muscarinic receptor activation is

the result of a direct agonist action by LAAM or an action to indirectly increase muscarinic receptor affinity. No precise mechanism is known.

b. The sympathetic nervous system

The action of LAAM was studied in animals pretreated with 6-OH dopamine, which makes the sympathetic nervous system nonfunctional except for the adrenal medulla and isolated chromaffin tissue (Thoenen and Tranzer, 1968). Chemical sympathectomy (SYMX) had a significant effect on the cardiovascular responses to LAAM.

The SYMX significantly increased the dose of LAAM required to decrease blood pressure. This indicates that the BP response to LAAM may involve an action on the sympathetic nervous system. However, it is possible that a significantly lower vascular tone in SYMX animals is responsible for the change in the BP response to LAAM. Therefore, the lack of effect of lower doses of LAAM on BP in SYMX animals cannot be taken as proof of involvement of the sympathetic nervous system in the mechanism for the BP response to LAAM. Analogous experiments to those in SYMX animals, involving C_1-spinal transected animals, have been done by others to investigate the contribution of an action on the sympathetic nervous system to the BP effects other narcotics. One such study found that morphine failed to decrease BP following C_1 transection (Evans et al., 1952). Similar data have been obtained for dextromoramide and fentanyl (Laubie et al., 1973). Later studies revealed that morphine, fentanyl, and dextromoramide decrease BP by a central action to depress sympathetic nervous system function (Laubie et al., 1974; Daskalopoulos et al., 1975). LAAM appears to be different from these compounds since it produced a BP decrease in SYMX animals. However, the possibility exists that a portion of the

hypotensive effect in intact animals may be due to an action on the sympathetic nervous system.

The effect of LAAM on HR in SYMX animals was different from that observed in intact animals. SYMX did not change the threshold dose but did decrease the magnitude of the bradycardia produced by LAAM. It might be argued that the lower HR in SYMX, as compared to intact animals, was responsible for the smaller negative chronotropic effect. However, the fact that the negative chronotropic response is less in SYMX animals, whether expressed as absolute HR change in beats per minute or as percent of control HR, would indicate that this probably is not the case. It appears that part of the bradycardia produced by LAAM in intact animals may be the result of an action by LAAM on the sympathetic nervous system. In contrast to the BP and HR responses to LAAM, the CF response to LAAM was not altered by SYMX. However, an action by LAAM on the sympathetic nervous system could contribute to the CF response to LAAM in intact animals (see later discussion).

The effects of LAAM on the sympathetic nervous system were examined. Before discussing the results of those experiments, let us consider the possible sites of action for LAAM on the sympathetic nervous system.

There are, in general, two sites at which LAAM could act to depress sympathetic nervous function, central and peripheral. Other narcotics produce cardiovascular responses via actions at central loci. Gomes and co-workers (1976) found a correlation between morphine-induced changes in norepinephrine turnover in the medulla and the bradycardia and hypotension produced by morphine in the rat. These changes were the

same as those produced by clonidine, an agent known to decrease BP and HR by decreasing sympathetic nerve activity (Schmitt et al., 1968). Dextromoramide and fentanyl also decrease sympathetic nerve activity and increase vagal tone by a central mechanism thereby decreasing BP and HR (Daskalopoulos et al., 1975; Schmitt et al., 1977b). Most recently, β-endorphin and [d-ala^2]-met-enkephalin have been shown to produce hypotension and bradycardia by a central mechanism of sympathetic nervous system depression (Schmitt et al., 1977a). These depressant effects by narcotics on the sympathetic nervous system can be completely antagonized by naloxone and it is the concensus that the primary mechanism for these effects involves the interaction of these agents with centrally located opiate binding sites.

In the periphery there are additional loci at which LAAM could act to decrease sympathetic function, i.e., the sympathetic ganglion, the nerve terminal or the neuroeffector. A majority of the reports in the literature indicate that morphine and other narcotics have little effect on sympathetic ganglionic transmission (Trendelenburg, 1957; Lees et al., 1973). Morphine has been reported to produce ganglionic blockade, but only at extremely high doses (Forbes and Dewey, 1976). There are several reports in the literature describing interactions of narcotics with the function of peripheral sympathetic nerve terminals. Among the best defined are the effects of narcotics at the sympathetic nerve terminals of the cat nictitating membrane (Trendelenburg, 1957). Narcotics interact with opiate binding sites on the sympathetic nerve terminals to decrease norepinephrine release from the terminals in this model system (Henderson et al., 1973). Similar effects at blood vessels have been suggested to contribute to the

cardiovascular actions of narcotics (Kayaalp and Kaymakcalan, 1966; Kennedy and West, 1967; Ward et al., 1972). The muscarinic cholinergic actions of LAAM also may produce effects at peripheral sympathetic nerve terminals. Muscarinic agonists can decrease electrically evoked release of norepinephrine from sympathetic nerve terminals (Vanhoute, 1974). More recent evidence indicates that presynaptic muscarinic receptors function as peripheral modulators at the sympathetic nerve terminals in the dog heart; muscarinic agonists were shown to decrease tonic norepinephrine release (Lavalee et al., 1978). Such an action by LAAM could contribute to the cardiovascular responses observed. The effect of atropine on the cardiovascular response to LAAM can also be explained by this hypothesis.

Data from SYMX animals suggest that a portion of the cardiovascular response to LAAM may be due to an action by the drug on the sympathetic nervous system. An investigation of the effects of LAAM on the sympathetic nervous system and the contribution of these effects to the cardiovascular response to LAAM was undertaken.

2. Specific Mechanisms for the Cardiovascular Actions of LAAM

a. The peripheral sympathetic nervous system

The model system chosen for the examination of the effects of LAAM on the peripheral sympathetic nervous system was the cat nictitating membrane preparation. Selection of this system was based on several factors. A system was desired which was discrete, well characterized, and in which the responses were easily measurable and quantifiable. The nictitating membrane fits these criterion. The innervation of the nictitating membrane smooth muscle is purely adrenergic fibers (Gardiner et al., 1962). Furthermore, the anatomical location and

arrangement of the preganglionic and postganglionic nerves to the nictitating membrane make it well suited for the study of drug effects. Unlike many sympathetic ganglia, the superior cervical ganglion has one preganglionic trunk and the postganglionic nerve which innervates the nictitating membrane is easy to identify and isolate. The ganglia involved in direct cardiovascular control, such as the stellate ganglion which innervates the heart, have many preganglionic and postganglionic branches associated with them. This makes it difficult to identify a specific pathway which can be shown to continue through the ganglion and which can be consistently obtained from animal to animal for study. These problems make such a system undesirable for a quantitative study. For these reasons it was felt that the nictitating membrane was a good system in which to examine the effects of LAAM. The use of cats was justified by data which demonstrated that LAAM had cardiovascular effects in the cat similar to those in the dog.

LAAM decreased the response of the nictitating membrane to preganglionic and postganglionic nerve stimulation. The data suggest that LAAM is not acting on the responses of nictitating membrane to stimulation by decreasing the responsiveness of the membranes to neurotransmitter; the responses to epinephrine were unchanged. Muscarinic receptors are not involved in the effect of LAAM on the sympathetic nervous system since atropine did not antagonize the effects of LAAM on the nictitating membrane responses. LAAM also was not acting at the sympathetic ganglion, since no differences were observed between its effects on preganglionic as opposed to postganglionic stimulation responses. Similar effects have been noted for other narcotics (Trendelenburg, 1957; Cairnie et al., 1961; Henderson et al., 1973; Lees et

al., 1973). The effect of LAAM appears to be at the sympathetic nerve terminal. Other narcotics decrease the release of norepinephrine from sympathetic terminals (Henderson et al., 1973; Kosterlitz and Waterfield, 1975). An important observation concerning the effects of LAAM was that the responses to low frequency stimulation (0.5-5.0 Hz) were preferentially inhibited. This is significant since tonic sympathetic nerve activity is known to be in the range of 0-5.0 Hz frequency (Rosenblueth, 1950). It suggests that LAAM may affect tonic sympathetic neurotransmission.

The lowest doses of LAAM required to significantly decrease the responses of nictitating membranes to sympathetic nerve stimulation, BP and HR were the same. This was the first direct evidence that LAAM affected sympathetic neurotransmission at the same doses at which it produced its cardiovascular actions. In addition, naltrexone antagonized both the actions of LAAM of the peripheral sympathetic nervous system and the cardiovascular actions of LAAM. This suggests that an action by LAAM on the peripheral sympathetic nervous system may contribute to the cardiovascular actions of LAAM. The interaction of LAAM with opiate binding sites appears to be involved.

b. Opiate binding sites, the sympathetic nervous system, and direct cardiac effects

Naltrexone completely antagonizes the BP response to LAAM. This finding suggests that LAAM interacts with opiate binding sites to produce the BP response. The nictitating membrane experiments indicate that the action of LAAM on the sympathetic nervous system involves opiate binding sites. The minimum doses required for the BP

and sympathetic nervous system effects are the same. These results suggest that LAAM may decrease BP by a depressant action on peripheral sympathetic nerve transmission which involves opiate binding sites. It has been reported that other narcotics depress the responses of isolated vascular beds to nerve stimulation (Ward et al., 1973). The depressant effects of morphine on the peripheral sympathetic nervous system have been suggested to contribute to its hypotensive action (Kayaalp and Kaymakcalan, 1966). The involvement of opiate binding sites in the actions of both LAAM and morphine makes it reasonable to suggest that LAAM and morphine share this mechanism of action, even though LAAM is a group II, and morphine a group I, narcotic. This mechanism of action would explain data discussed earlier which suggest that LAAM decreases BP through combined actions on the heart and vasculature. Although LAAM has depressant actions on the heart which do not involve opiate binding sites (see later discussion), impairment of sympathetic neurotransmission could result in decreases in HR and cardiac output, as well as decreases in peripheral resistance. Depressed sympathetic neurotransmission also would impair the ability of the baroreceptor reflexes to compensate for the depressant actions of LAAM on the heart which decrease cardiac output (see later discussions). LAAM may also act centrally to impair baroreceptor reflex function. Other narcotics such as fentanyl and dextromoramide act centrally through an interaction with opiate binding sites to affect sympathetic nerve activity and in this way decrease BP (Laubie et al., 1974; Daskalopoulos et al., 1975; Laubie et al., 1977b). However, no attempt was made to examine this possibility for LAAM.

If, as suggested above, an action on the sympathetic nervous system is a primary mechanism which contributes to the BP response to LAAM, why does LAAM decrease BP in SYMX + VAGOT animals? There are several explanations. LAAM could act directly on vascular smooth muscle to decrease BP. However, Lee and Berkowitz (1977), while examining the effect of LAAM on Ca^{+2}-dependent contractions of isolated aortic strips, found no evidence for an effect of LAAM on resting vessel tension. In addition, naltrexone completely antagonizes the effect of LAAM on BP, presumably by antagonizing neurally mediated effects of LAAM. This suggests that any direct effects LAAM has on the vasculature contribute minimally to the BP response and are compensated for by neural influences in naltrexone pretreated animals. A second explanation for the BP response in SYMX + VAGOT animals is that the decrease in HR and CF caused by the highest dose of LAAM could result in a substantial decrease in cardiac output. Since the baroreceptor reflex has been rendered ineffective by SYMX + VAGOT, compensating increases in vascular resistance to maintain BP cannot be made. As a result, BP falls. The total blockade of the BP response to LAAM by naltrexone could then be explained by the fact that although naltrexone cannot block the direct effects of LAAM on HR, CF and presumably cardiac output (see later discussion), naltrexone abolishes the impairment of baroreceptor reflex function and BP can be maintained. The BP response to LAAM in intact animals, appears to be the result of an action to depress sympathetic neurotransmission to the heart and vasculature which involves opiate binding sites and direct cardiac actions of LAAM to decrease HR, CF, and cardiac output which occur even when neural influences on the heart have been eliminated (see later discussion).

Opiate binding sites appear to be involved in the HR response to LAAM. Naltrexone pretreatment significantly attenuated, but did not completely block, the bradycardia produced by LAAM. Two theories could explain this data. One theory is that the naltrexone antagonism of the HR response to LAAM is the result of nonspecific mechanisms, not direct interactions of the two drugs at opiate binding sites. Another theory is that there are two independent mechanisms which combine to cause the HR response to LAAM. One mechanism would involve opiate binding sites and the other would not. I prefer the second theory for several reasons. First, LAAM appears to interact with opiate binding sites to depress the function of the sympathetic nervous system. It seems unlikely that this same mechanism does not also contribute to the HR response to LAAM. Secondly, the HR responses to LAAM in SYMX + VAGOT and SYMX animals were similar to each other and less than in intact animals. This would suggest that part of the bradycardia produced by LAAM is the result of an action by LAAM on the sympathetic nervous system. When this is considered together with the fact that LAAM depresses peripheral sympathetic neurotransmission by a mechanism antagonized by naltrexone and that part of the HR response to LAAM is also antagonized by naltrexone, it does not seem unreasonable to suggest that part of the negative chronotropic action of LAAM is the results of an action by LAAM on the sympathetic nervous system which involves opiate binding sites. The fact that HR is decreased in SYMX + VAGOT animals suggests the possibility that LAAM can decrease HR by direct or muscarinically mediated actions on the heart. Furthermore, the threshold dose for these negative chronotropic actions is the same as the threshold

dose for the effects by LAAM on the sympathetic nervous system which involve an interaction of LAAM with opiate binding sites. Thus, the HR response to LAAM could be the results of an action of LAAM on the sympathetic nervous system which involves the interaction of LAAM with opiate binding sites in combination with direct depressant actions on the heart.

To satisfactorily explain the effects of naltrexone on the HR response to LAAM, the direct depressant actions on the heart to decrease HR discussed above could not involve the interaction of LAAM with opiate binding sites. In vitro studies indicate that LAAM can produce negative chronotropic effects which do not involve opiate binding site interactions (Stickney, 1977a). Therefore, it is likely that a part of the negative chronotropic response to LAAM in vivo does not involve opiate binding site interactions and data from naltrexone experiments support this. A question still remains, however, with regard to the mechanisms for these cardiac effects. Previous discussions have entertained explanations for the actions of LAAM on HR which involve muscarinic receptors. However, in vitro data suggest that only part of the negative chronotropic response to LAAM requires muscarinic receptor activation (Stickney, 1978a). The mechanism for the the negative chronotropic action which is not antagonized by atropine is unknown. Other drugs are known to have direct actions on the heart. The most familiar drugs in this category are the antiarrhythmics which appear to have membrane stabilizing properties of an undetermined mechanism. Several of these agents, including quinidine and lidocaine, have been shown to decrease HR in normal animals at doses which are considered to produce the therapeutic membrane stabilizing effects

(Harrison et al., 1963; Austen and Moran, 1965; Parmley and Braunwald, 1967). Recently, similar membrane-stabilizing effects have been suggested for a structural analogue of LAAM, propoxyphene (Holland and Steinberg, 1979). Perhaps part of the direct cardiac action of LAAM to decrease HR is the result of a similar effect.

The CF response to LAAM also appears to involve the interaction of LAAM with opiate binding sites. Naltrexone antagonized, but did not completely block the HR response to LAAM. I propose that LAAM decreases CF by a mechanism similar to that proposed for the HR response, that is by a combination of effects on the sympathetic nervous system which require opiate binding site interaction and other effects on the heart which do not. Decreased coronary blood flow as a result of lowered BP and/or coronary vasoconstriction could lead to a decrease in CF. However, LAAM has direct negative inotropic effects on isolated atria (Stickney, 1977b). These inotropic effects of in vitro are not antagonized by naloxone (Stickney, 1977b). Similar cardiac actions by LAAM in vivo would explain why naltrexone does not completely antagonize the CF response. The data from SYMX + VAGOT animals indicate that LAAM has direct actions on the heart in vivo to decrease CF. However, the effects of LAAM on CF in SYMX and SYMX + VAGOT animals are similar to each other and not different from those observed in intact animals. These data suggest that the effect of LAAM on CF in intact animals is not due to an action of the sympathetic nervous system. How can this be explained in light of the fact that naltrexone antagonizes the CF response to LAAM? We know that LAAM can decrease HR and CF by direct actions on the heart. You will recall that peripheral resistance increases following LAAM, probably due to reflex increases in sympathetic

nerve activity in response to the decrease in cardiac output. However, LAAM also appears to depress peripheral sympathetic nerve function. I propose that in intact animals, the effect of the reflex partially compensates for the direct effect of LAAM on CF. However, this compensation would be greater if LAAM did not depress peripheral sympathetic function, as is the case in naltrexone pretreated animals. Perhaps the CF response in SYMX + VAGOT animals appears to be the same as in intact animals because SYMX not only eliminates the actions of LAAM on the sympathetic nervous system but also eliminates any compensatory effects of the sympathetic nervous system. These two effects would cancel each other and SYMX would not appear to affect the CF response to LAAM. The larger magnitude of the HR response to LAAM allows for clear separation of the effects on the sympathetic nervous system and direct cardiac effects.

The direct action of LAAM on the heart to decrease CF has been examined *in vitro*. These studies have shown that the negative inotropic response to LAAM can be antagonized by increasing the calcium concentration in the extracellular medium. This has led to the suggestion that LAAM acts as a calcium antagonist (Stickney, 1978b). Calcium antagonism also has been suggested as a mechanism contributing to the analgesic actions of morphine (Way *et al.*, 1978). However, a precise mechanism of action has not been determined. Other drugs known to have direct actions on the heart, such as antiarrhythmics which stabilize cardiac membranes, also can decrease CF at doses which produce antiarrhythmic protection (Harrison *et al.*, 1963; Austen and Moran, 1965; Mixter *et al.*, 1966; Parmley and Braunwald, 1967). Both of the above mechanisms and/or other mechanisms may contribute to the CF response to LAAM.

In summary, opiate binding sites appear to be involved in the mechanisms for the cardiovascular actions of LAAM. The BP response to LAAM appears to be the result of interactions with opiate binding sites and probably involves depression of the peripheral sympathetic nervous system. The possibility of a central action on sympathetic nervous system exists but was not evaluated. The effect of LAAM on the sympathetic nervous system contributes to the effects of LAAM on HR and CF. Part of the bradycardia appears to involve muscarinic receptors via an unknown mechanism. In addition, direct actions on the myocardium appear to contribute to the effects of LAAM on HR and CF. These direct cardiac actions do not involve interactions with opiate binding sites.

With these mechanisms of action for LAAM in mind, let us now consider the greater potency of nor-LAAM as opposed to LAAM and dinor-LAAM in producing hypotensive and negative chronotropic effects. Nickander and co-workers (1974) found that nor-LAAM and dinor-LAAM were approximately 10 times more potent in inhibiting contractions of the electrically stimulated guinea pig ileum than was LAAM. These actions are thought to be due to an opiate binding site interaction since they are antagonized by naloxone. However, in analgesia testing _in vivo_, nor-LAAM was approximately 10 times more potent than LAAM or dinor-LAAM (Smits, 1974). It would appear that, _in vitro_, nor-LAAM and dinor-LAAM are more potent opiate agonists than LAAM, but, _in vivo_, nor-LAAM is considerably more potent than either LAAM or dinor-LAAM as an opiate agonist. I suggest that nor-LAAM and dinor-LAAM decrease BP and HR by the same mechanisms as LAAM. If previous findings concerning _in vivo_

potency of LAAM and nor-LAAM at opiate binding sites apply with regard to cardiovascular actions, this could explain why nor-LAAM is more potent than LAAM. LAAM, nor-LAAM and dinor-LAAM were equipotent *in vivo* with regard to their actions on CF. This suggests that opiate binding sites do not play an important role in the CF response to nor-LAAM. The possibility exists, however, that the CF effects of low doses of nor-LAAM involve opiate binding site interactions but are small and not detected. The relative *in vivo* potency of LAAM and its metabolites in decreasing CF agree well with *in vitro* data. *In vitro*, nor-LAAM was equipotent with LAAM and dinor-LAAM with regard to negative inotropic effects (Stickney, 1978b). Therefore, the fact that LAAM and dinor-LAAM are less potent than nor-LAAM as hypotensive and negative chronotropic agents could be explained by the theory that LAAM and its metabolites decrease BP and HR, at least in part, by an interaction with opiate binding sites. The importance of opiate binding sites in the CF effects of nor-LAAM is not clear.

In closing, I would like to return to the earlier classification of narcotics with regard to their cardiovascular actions. You will recall that morphine, dextromoramide, and fentanyl belong to group I. Several investigators have shown that these agents produce their cardiovascular effects via actions on the autonomic nervous system involving opiate binding site interactions (Grundy, 1971; Laubie *et al.*, 1974; Daskalopoulus *et al.*, 1975; Laubie *et al.*, 1977b). A central site of action has been identified for dextromoramide and fentanyl (Laubie *et al.*, 1974; Daskalopoulos *et al.*, 1975; Laubie *et al.*, 1977b) and both central and peripheral sites of action have been suggested for the effects of morphine (Kayaalp and Kaymakcalan, 1966; Mansour *et al.*,

1970; Lowenstein et al., 1972). No direct effects on the heart are observed after morphine, dextromoramide, or fentanyl at doses which decrease BP and HR if the autonomic influences on the heart are removed (Lowenstein et al., 1969; Laubie et al., 1974).

Methadone, meperidine, LAAM and the metabolites of LAAM, on the other hand, belong to group II. Methadone and meperidine have been shown to have direct cardiac effects at the lowest doses at which they decrease BP and HR (Chen, 1948; Sugioka et al., 1957). The present study shows that LAAM has direct cardiac actions in vivo at doses which decrease BP and HR. The data also suggest that nor-LAAM and dinor-LAAM may have this property. Finally, the effects of LAAM, nor-LAAM and probably dinor-LAAM, on the cardiovascular system involve other mechanisms in addition to interactions with opiate binding sites.

These data suggest that important differences exist between the in vivo cardiovascular actions of the two groups of narcotics. Group I agents appear to produce their cardiovascular actions primarily by interacting with opiate binding sites to alter the function of the autonomic nervous system. No direct effects on the heart are observed at the lowest doses which decrease BP and HR. In contrast to this LAAM and other group II agents have direct depressant effect on the heart, in addition to actions shared by group I compounds, at the lowest doses which decrease BP and HR. This is significant because direct cardiac actions are often thought to be associated with doses much higher than those required for opiate binding site interactions. These data also suggest that the common clinical treatment in the case of narcotic overdose, narcotic antagonist administration, may not completely antagonize the cardiovascular actions of LAAM and nor-LAAM.

SUMMARY AND CONCLUSIONS

The purpose of this investigation was to examine, and determine mechanisms for, the *in vivo* effects of LAAM, nor-LAAM and dinor-LAAM on the cardiovascular system. The present study demonstrates that LAAM, nor-LAAM and dinor-LAAM can significantly decrease BP, HR and CF. LAAM and dinor-LAAM appeared to be less potent than nor-LAAM with regard to hypotensive and negative chronotropic activity. Data from naltrexone pretreated animals suggest that the greater potency of nor-LAAM probably is the result of its greater potency at opiate binding sites *in vivo*. All 3 drugs appeared to be equipotent with regard to their effects on CF.

LAAM and practolol both caused significant decreases in cardiac output and BP. However, the BP decrease caused by LAAM, with a comparable decrease in cardiac output, was greater than that caused by practolol. The data suggest that, in addition to the effect of LAAM to decrease cardiac output, vascular changes caused by LAAM also contribute to the BP response to LAAM.

Experiments in vagotomized (VAGOT) animals suggested that vagal tone did not contribute significantly to the mechanism of action for LAAM. This was surprising since the negative chronotropic action of LAAM *in vivo* is antagonized by atropine. In an attempt to identify a mechanism for the effects which involve muscarinic receptor activation,

the anticholinesterase activities of LAAM, nor-LAAM and dinor-LAAM were assessed. All 3 drugs had significant anticholinesterase activity in preparations of purified acetylcholinesterase, purified butyrylcholinesterase, guinea pig heart homogenate and guinea pig plasma. However, it was determined that anticholinesterase activity probably was not the primary mechanism for the muscarinic receptor mediated negative chronotropic effects. Experiments in sympathectomized (SYMX) animals suggested that an action by LAAM on the sympathetic nervous system might contribute to the BP and HR response to LAAM. SYMX had no effect on the CF response to LAAM. LAAM decreased BP, HR and CF in SYMX + VAGOT animals. These data were not significantly different from those obtained in SYMX animals. The data suggest that LAAM has significant depressant effects on heart in vivo and that the minimum dose required for these cardiac effects is the same as the minimum dose required for significant decreases in HR and CF in intact animals.

LAAM significantly attenuated the responses of the nictitating membranes to low frequency (0.5-5 Hz) sympathetic nerve stimulation and the data suggested that the site of action was the nerve terminals. The minimum doses required for the effects of LAAM on the responses to nerve stimulation and on BP and HR were the same. Naltrexone completely blocked the effects of LAAM on the nictitating membrane responses and BP, and partially antagonized the effect of LAAM on HR. These data suggest that an interaction with opiate binding site by LAAM, resulting in depression of peripheral sympathetic nervous system function, may be causally related to the BP and, in part, the HR response to LAAM.

Experiments in naltrexone pretreated animals showed that naltrexone completely antagonized the effect of LAAM on BP. The HR response to LAAM was antagonized, but not completely blocked by naltrexone pretreatment. Further investigation suggested that the antagonism was incomplete because the HR response to LAAM is partially due to an interaction with opiate binding sites and partially due to cardiac actions of LAAM. The CF response to LAAM was partially antagonized by naltrexone. The exact nature of the antagonism was not clear.

In conclusion, LAAM, nor-LAAM and dinor-LAAM caused significant decreases in BP, HR, and CF in vivo. Nor-LAAM appeared to be more potent in decreasing BP and HR than LAAM and dinor-LAAM, but all 3 drugs appeared to be equipotent in decreasing CF. The differences between the cardiovascular effects LAAM, nor-LAAM and dinor-LAAM appear to be quantitative and not qualitative. The data suggest that nor-LAAM appears to be more potent because of its greater potency at "opiate" binding sites in vivo. The effect of LAAM on BP appears to be due to an action through opiate binding sites to depress the function of the peripheral sympathetic nervous system and depressant actions of the heart. This leads to impairment of baroreceptor reflex function and decreased cardiac output. The effect of LAAM on HR appears to be due to a combination of the effects through opiate binding sites on the peripheral sympathetic nervous system and cardiodepressant effects. A portion of the cardiodepressant effects appears to be mediated by muscarinic cholinergic receptor on the heart via an unknown mechanism. The remainder of the cardiodepressant effects appear to be the result of

direct actions of LAAM on the myocardium. Another possible contributing mechanism to the BP and HR effects of LAAM is an effect through opiate binding sites on the central nervous system to depress the sympathetic nervous system. However, the present study did not evaluate this possibility. The effects of LAAM on CF appear to be primarily the results of direct depressant effects on the heart, although actions involving opiate binding sites probably also are involved. The most important finding is that LAAM appears to have direct cardiac actions *in vivo* and the minimum dose required for these direct actions is the same as the minimum doses required for the effects of LAAM on BP and HR which involve opiate binding site interactions.

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