STUDIES ON THE EFFECTS OF p-CHLOROMERCURIBENZOIC ACID AND OF METHOXAMINE ON ATRIOVENTRICULAR TRANSMISSION

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ABSTRACT

Blockade of atrioventricular transmission by p-chloromercuribenzoic acid (p-CMB) and potentiation of this effect by methoxamine were studied.

p-CMB (20-80 mg/kg) caused atrioventricular (AV) nodal block in pentobarbital-anaesthetized dogs. Methoxamine (0.4 or 1.6 mg/kg) given before p-CMB increased the incidence of AV-nodal block. This potentiating effect of methoxamine was not due to reflex effects which alter sympathetic and vagal tone to the heart.

The pressor response to methoxamine was not correlated with the incidence of AV-nodal block. This indicated that factors other than the pressor response are involved in the potentiating effect of methoxamine.

p-CMB (20-80 mg/kg) lowered the systemic arterial blood pressure. p-CMB (20 mg/kg) caused a significantly greater fall in the methoxamine-pretreated dogs. The fall in blood pressure was significantly greater and the final level of the blood pressure was significantly lower in those dogs subsequently developing AV-nodal block after p-CMB (20 mg/kg).

This was not the case in dogs without vagal or sympathetic innervation to the heart.

There was no relationship between changes in heart rate from either methoxamine or p-CMB and the incidence of AV-nodal block. This suggested that the heart rate is not involved in the potentiating effects of methoxamine.

p-CMB (20-80 mg/kg) significantly lengthened the P-R interval without causing any change in the duration of the QRS complex on the electrocardiogram. Methoxamine (0.4 or 1.6 mg/kg) did not significantly alter either the P-R interval or the QRS complex. However a significantly greater increase in the P-R interval in response to p-CMB (20 mg/kg) occurred in dogs given methoxamine (0.4 or 1.6 mg/kg) beforehand.

Methoxamine (0.4 mg/kg) or p-CMB (5-40 mg/kg) prolonged the functional refractory period of atrioventricular transmission (FRP) and increased the conduction time of atrioventricular transmission (CT). When the increase in blood pressure due to methoxamine was prevented the prolongation of the FRP and increase in CT was less. Mechanically induced increases in blood pressure significantly prolonged the FRP but did not increase the CT.

It was concluded that p-CMB caused AV-nodal block by depressing atrioventricular transmission. This depressant effect of p-CMB was potentiated by methoxamine

in two ways. First, by the direct depressant effect of methoxamine and secondly, by the rise in blood pressure caused by methoxamine.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

SECTION I

INTRODUCTION

A. HISTORICAL REVIEW

1. The Cardiac Toxicity of Organic Mercurials

In 1922 Salant and Kleitman (1) demonstrated that organic and inorganic mercurials were toxic to the cardiovascular system. They found that the intravenous administration of acetate, succinate or benzoate of mercury to cats, dogs, and rabbits, produced a marked fall in the blood pressure followed by respiratory depression. They observed also (2) that when mercuric chloride was given to the isolated perfused turtle heart the amplitude and frequency of contraction were decreased. This compound also produced what the authors termed "delerium cordis" (ventricular fibrillation). Additional observations (3) in the isolated frog heart indicated that mercuric chloride acted upon the conduction system and the musculature, producing a decrease in the force and frequency of contractions, followed by extrasystoles and heart block. Atropinization prevented the decrease in heart rate produced by small doses of mercuric chloride, but did not prevent the decrease in the force of contraction, extrasystoles or heart block caused by large doses.

There has been some disagreement as to the role of the vagus in the cardiac toxicity of the organic mercurials. Jackson (4) observed that the organic mercurial diuretic mersalyl produced ventricular fibrillation in dogs. Death,

however, occurred from respiratory failure before the onset of ventricular fibrillation if the vagal inhibition of the heart was removed by vagotomy or atropine. He postulated therefore, that the organic mercurials acted primarily on the heart through the mechanism of vagal stimulation and secondarily on the respiratory center. However in contrast Salant and Kleitman (1) showed that atropinization did not alter the cardiac toxicity of the organic or inorganic mercurials. Additional findings by Barker et al (5) indicated that vagotomy or spinal section did not alter the cardiac toxicity of the organic mercurials in dogs.

McCrea and Meek (6) showed that mercuric chloride caused a characteristic sequence of toxic events as observed on the electrocardiogram of cats and dogs. There was an early transient acceleration of the heart rate, followed by a gradual bradycardia and increases in the P-R interval and duration of the QRS complex. These changes were followed by bundle branch or atrioventricular nodal (AV) block, ventricular tachycardia and ventricular fibrillation. They concluded that the sino-atrial node (SA) was more susceptible to the action of mercuric chloride than was the AV-node, and that the latter was more susceptible than the remainder of the specialized conducting system.

In contrast (3,5) the organic mercurials have been shown to produce ventricular asystole while the atria continued

beating with regular sinus rhythm in both dog and frog hearts. This observation is supported by other authors (7,8,9) who have shown that in the dog the atria are more resistant to the organic mercurials than the ventricles.

In an extensive study Barker et al (5) showed that the cardiac toxicity of both organic and inorganic mercurials followed similar patterns, namely depression of the T wave, runs of extrasystoles and ventricular fibrillation. These observations were made in both unanaesthetized and barbital-anaesthetized dogs. In another study Pines et al (8) found that the organic mercurials increased the P-R interval and the duration of the QRS complex, and caused complete heart block followed by ventricular ectopic beats.

DeGraff and Lehman (7) showed that the presence of the organic residue of the organic mercurial increased the lethal dose; however when the dose was increased the same sequence of toxic events occurred. The cardiac toxicity of the organic mercurials was also reduced by moderate doses of adrenaline (10,11), whereas anoxia of the heart muscle increased the cardiac toxicity (8).

Farah and Mook (12) showed that the organic mercurials mersalyl and esidron acid in toxic doses decreased conduction velocity and electrical excitability, and increased the "effective refractory period" of both the atria and ventricles in dogs. The changes in refractory period occurred with much

larger doses than the changes in either excitability or conduction velocity. A more recent study (13) showed that mersalyl slowed the heart rate, shortened the transmembrane action potential, and decreased the contractility of the isolated perfused guinea pig atria.

Farah et al (9) showed both quantitative and qualitative differences in the cardiac toxicity of various organic mercurials. p-Chloromercuribenzoic acid (p-CMB), a non-diuretic organic mercurial, produced different electrocardiographic changes than the diuretic organic mercurials or mercuric chloride. p-CMB did not cause any changes in the duration of the QRS complex, while the diuretic organic mercurials and mercuric chloride caused a widening of the QRS complex. p-CMB caused death by ventricular asystole whereas the other mercurials caused death by ventricular fibrillation. However both p-CMB and the diuretic organic mercurials increased the P-R interval. These authors postulated that p-CMB selectively inhibits atrioventricular (A-V) conduction without affecting intraventricular conduction processes.

Further studies on the properties of p-CMB were carried out by Kessler, Lozano, and Pitts (14). They showed that 33.3% of an initial dose of Hg^{197} labelled p-CMB was found in the heart after three hours. The plasma clearance of labelled mercury was 10 cc/min, and 40% of the administered intravenous

dose was eliminated in three hours. p-CMB was also found to be highly bound to plasma proteins. Mercuhydrin and other organic mercurials have also been shown to be highly bound to plasma protein (15,16).

A number of reports (10,12,17,18,19,20) have shown that the cardiotoxic effects of the organic mercurials are prevented or abolished by the dithiol dimercaprol and by the monothiols cysteine, glutathione, and thioglycollic acid. Other studies (21,22,23,24) have shown that the organic mercurials inhibit sulfhydryl-containing enzymes which are intimately involved in carbohydrate, fat, and protein metabolism. On the basis of these observations a number of authors (12,16,17) postulated that the cardiac disturbances produced by the organic mercurials are due to the inhibition of one or more of these sulfhydryl-containing enzyme systems.

2. The Cardiac Effects of Methoxamine

In 1948 Huort, Randall and DeBeer (25) reported that methoxamine, a sympathomimetic amine, produced a sustained pressor response in dogs. However, Melville and Lu (26) showed that methoxamine differed from other sympathomimetic amines in that its cardiac actions are depressant in nature, while the majority of other sympathomimetic amines have a stimulatory effect on the heart.

Nathanson (27) showed that the intravenous

administration of methoxamine in man caused a slowing of the heart rate which was abolished or prevented by atropine indicating that the bradycardia was mediated reflexly. Other authors (28,29) showed that the mechanism by which methoxamine caused cardiac slowing was mainly due to stimulation of the carotid sinus and aortic arch baroreceptors provoked by the rise in systemic blood pressure. A slight bradycardia, however, persisted after carotid sinus and aortic arch denervation which was not due to a direct action of methoxamine on the cardioinhibitory center of the medulla oblongata nor on the sino-atrial node. The mechanism of this slight bradycardia is uncertain. One study (29) indicated that this bradycardia was due to stimulation of the coronary and pulmonary receptors involved in the Bezold-Jarish reflex, while another study (28) suggests that it is due to activation of stretch receptors in the ventricular wall.

Gilbert et al (30,31) showed that methoxamine, in contrast to adrenaline, increased the threshold for ventricular excitability and decreased cardiac irritability. The ventricles of open chest dogs were stimulated at varying intervals after a regular driving stimulus. Adrenaline produced abnormal or multiple responses to these single ventricular test stimuli, while methoxamine did not. In addition they found that methoxamine did not produce ectopic pacemakers, or spontaneous extrasystoles whereas they were produced by adrenaline. In

another study (32) adrenaline was shown to cause ventricular arrhythmias in the chloroform- or cyclopropane-sensitized hearts, whereas such an effect is not observed with methoxamine; indeed methoxamine prevents cyclopropane-adrenaline arrhythmias.

Gilbert et al (30) have shown that methoxamine causes slowing of atrioventricular conduction in association with decreased excitability in the hearts of vagotomized dogs. The "absolute refractory period" of the ventricle and the duration of the ventricular action potential recorded extracellularly were lengthened by intravenous administration of methoxamine. Atrioventricular conduction time was prolonged while ventricular and atrial conduction were not altered.

Melville and Lu (26) showed that methoxamine decreased the amplitude of contraction in the isolated rabbit heart. Methoxamine had a marked negative inotropic and a slight negative chronotropic effect on the isolated perfused guinea pig heart (33,34). In addition methoxamine has been shown to have a negative inotropic effect in vagotomized dogs (35,36, 37). Goldberg et al (38) found that methoxamine produced a slight decrease in the myocardial contractile force of human subjects.

West and his co-workers (39,40,41,42,43) found that moderate to large doses of methoxamine (12-25 ug/kg) injected into the coronary arteries of dogs caused depression of myocardial contractility, while small doses had no effect.

However, methoxamine in large or small doses administered in this way did not cause any change in the heart rate.

A number of authors (44,45,46) reported that there is a direct relationship between phosphorylase activity and contractile force in the isolated rat heart and the dog heart *in situ*. Adrenaline increased both the contractile force and the phosphorylase activity, but methoxamine had no effect on either. Hashimoto *et al* (47) reported that methoxamine reduced the oxygen consumption in the isolated fibrillated dog heart, and suggested that this represented inhibition of cardiac metabolism.

Imai (48) studied the effects of methoxamine on the transmembrane potential of guinea pig ventricular muscle. He found that methoxamine caused a reduction in the maximal rate of rise and a prolongation in the duration of the action potential, but did not affect its magnitude. He suggested that methoxamine caused a depression of the selective increase in sodium permeability of the cell which causes the rising phase of the action potential. The prolongation of the action potential was attributed to an inhibition of cardiac metabolism.

Imai, Shigei, and Hashimoto (49,50) studied both the cardiac actions of large doses of methoxamine, and the antagonistic action of methoxamine to adrenaline, in the dog heart lung preparation. Methoxamine caused a marked negative inotropic action and a slight decrease in the heart rate. Pre-

treatment of the animal with reserpine abolished the chronotropic response but did not modify the inotropic response to methoxamine. Methoxamine was also shown to abolish both the positive inotropic and chronotropic actions of adrenaline. It was therefore suggested that the decrease in the heart rate induced by methoxamine was primarily due to its antagonistic action of the effects of intrinsic catecholamines, which may be released in small quantities to maintain the normal heart rate and contractility. It was suggested further that in view of the similarity in the chemical structures of adrenaline and methoxamine, it may be possible that the specific antagonism takes place at the same receptor site. Therefore it was postulated that methoxamine is a beta-adrenergic blocking agent. The negative inotropic action of methoxamine was ascribed to a direct cardiac effect independent of beta-adrenergic blockade.

3. Atrioventricular Transmission

Hearing in 1910 (51) attributed the main delay in the passage of impulses between the atrium and ventricles to the AV-node. He stimulated the isolated perfused rabbit heart above and below the AV-node and recorded the ventricular contractions. He found that the conduction time was four times longer when the heart was stimulated above the AV-node than when it was stimulated below the node. He therefore attributed a portion of the delay in conduction between the atrium and

ventricles to the AV-node.

Since that time there have been a number of suggestions as to the exact location of this delay in transmission, and to the mechanism by which it takes place. Erlanger and Blackman (52), and Eyster and Meek (53) showed that the AV-node was involved in the conduction of impulses from the atria to the ventricles by cutting the AV-node.

Erlanger in 1912 (54) postulated that the greatest part of the delay in transmission of an impulse from the atria to the ventricles occurred at the junction between the atrial and nodal fibers. A later study by Osborne et al (55) also indicated that the normal delay in atrioventricular conduction in the dog occurred in the atrial portion of the AV-node. They found that the time interval between the application of a stimulus and the appearance of the QRS complex on the electrocardiogram was greater when the AV-node was stimulated than when the stimulus was applied to the bundle of His. They also stimulated various regions of the AV-node while recording the ventricular response. From these experiments they concluded that the greatest delay in conduction occurred in the atrial portion of the AV-node.

Scher et al (56) also showed that the region between the atrium and the AV-node has the slowest conduction velocity. Using multiple extracellular electrodes in open chest dogs, they calculated that the conduction velocity within the AV-node

was double that at the atrial margin of the node.

Hoffman et al (57) and de Carvalho (58) utilized a slightly different technique and showed that almost all of the atrioventricular delay is localized in a narrow zone extending the full width of the AV-node, approximately 1 mm across and located near the junction of the atrium and the AV-node. They recorded simultaneously from the atrium, AV-node and the bundle of His with multiple intracellular microelectrodes inserted into isolated rabbit hearts. When they stimulated the atrium the action potentials recorded from fibers between the atrial portion of the AV-node and the bundle of His showed a gradual transition in shape, indicating a delay in conduction. Further studies by de Carvalho (59,60) indicated that the shape of the intracellular action potential was different in various regions of the AV-node of the isolated rabbit heart. On the basis of this he suggested that there were three distinct physiological regions in the AV-node. He designated these regions as the atrial portion of the AV-node, the atrionodal boundary (previously described as the atrionodal junction), and the lower node located near the bundle of His. The AV-node is described as being a complex of these three zones with the slowest propagation velocity occurring in the atrionodal boundary.

Cranefield et al (61) showed that acetylcholine caused

failure of impulse transmission in the fibers of the atrial margin of the AV-node and not in fibers of the AV-node adjacent to the bundle of His of the isolated rabbit heart. However, conflicting results were obtained by Pruitt and Essex (62). They showed that AV-nodal block produced by vagal stimulation occurred in the AV-node itself and not at the atrial or bundle margins of the AV-node. External needle electrodes were placed in various portions of the AV-node, atrium, bundle of His, and the bundle branches of bovine hearts. They noted that vagal stimulation was attended by a prolonged P-R interval on the electrocardiogram, but the duration of the potentials recorded from the atrionodal junction did not change. They therefore concluded that the delay in transmission occurs at a site in the AV-node closer to the ventricles than the atrium. However these discrepancies could be attributed to species differences or the distribution of the vagus nerve.

A different concept was advanced by Alanis et al (63, 64). They postulated the existence of two functional discontinuities in the atrioventricular conducting system. They suggested that delay in conduction takes place at two sites, one located between the atrium and the proximal segment of the AV-node, and the other between the distal part of the AV-node and the bundle of His. They stimulated the atrium and recorded electrograms from the bundle of His and the AV-nodal

region of the isolated perfused dog heart. The electrograms recorded at the atrium, AV-node, bundle of His, and ventricle revealed four distinct action potentials. When the heart was stimulated at low frequencies the interval between the atrial and the AV-nodal action potentials was greater than the interval between the AV-nodal potential and the bundle of His potential. However when the frequency of stimulation was increased the interval between the AV-nodal potential and the bundle of His potential increased and finally disappeared while the interval between the atrial potential and the AV-nodal potential remained constant. The interval between the atrial and AV-nodal potential was seen to increase only with very high stimulus frequencies. They suggested that the most critical region where atrioventricular transmission is delayed is located either somewhere within the AV-node or between the AV-node and the bundle of His. These findings therefore are in direct contrast to the hypothesis that the most critical region for the delay in atrioventricular transmission is the junction of the atrium and the AV-node.

A number of theories have been put forth to account for the mechanism of delay in atrioventricular transmission. Gilson (65) postulated an excitation time theory to account for the normal delay in atrioventricular conduction in the turtle heart. He suggested that fibers in the AV-node have an unusual requirement for stimulus duration and are activated only during

the repolarization of adjacent atrial fibers. Therefore the AV-node is activated by atrial repolarization and the time required for this activation is responsible for the delay in transmission. Grant (66) suggested that the AV-node is a continuous oscillator which fires only when the atrial impulses coincide with the nodal oscillations.

Scher et al (56) postulated three possible mechanisms for first degree and complete AV-nodal block. Firstly it is possible that some electrical mechanism is involved in the bridge of atrial and nodal fibers which is different from other cardiac tissues. Secondly, it is possible that the atrionodal junction may have action potentials of very long duration. The third possibility is that some ionic permeability changes may persist after the action potential of the cells in the atrionodal region of the AV-node has taken place. They obtained action potentials from small extracellular electrodes placed at various sites along the atrioventricular conduction pathway of the isolated perfused dog heart. From the shape of these action potentials, and because of the ability to achieve retrograde stimulation of the heart, they suggested that atrioventricular conduction involves a continuous electrical conduction of impulses which does not involve any synapses.

Hoffman and his co-workers (57,61,67,68), utilized intracellular microelectrodes to study the mechanism of atrioventricular delay. They inserted these microelectrodes

into the region of the AV-node of the perfused rabbit heart. The action potentials recorded from single cells in the region of the atrial portion of the AV-node showed a low resting potential, slow diastolic depolarization, and slow upstroke and low amplitude, and one or more notches or steps on the rising phase. These results indicated that the normal atrioventricular delay results from slow conduction within the AV-node rather than from refractoriness of the nodal tissue, or some synapse-like delay in the junction of the nodal fibers with the atrium or bundle of His. They suggested that the normal atrioventricular delay results from a very slow spread of excitation over a short distance which may be decremental in nature. The term decremental conduction is taken to mean that the action potential diminishes progressively in both amplitude and rate of depolarization. As a result the efficacy of the action potential as a stimulus to adjacent regions is continuously reduced. These findings are in agreement with the results obtained by Scher (56) who used extracellular electrodes. Furthermore, de Carvalho (59,60) showed that decremental conduction takes place only in the atrionodal boundary of the AV-node, whereas in the rest of the AV-node conduction is of the all or none type.

The failure of atrial electrical activity to be propagated to the ventricles was also studied by Hoffman et al (69). They used both intracellular microelectrodes in the

isolated rabbit heart, and local electrograms from the dog heart *in situ*. The shape of transmembrane potentials recorded from single AV-nodal fibers of the isolated rabbit heart indicated that block of conduction in the AV-node takes place as a result of decremental conduction. However in the dog heart *in situ* local electrograms revealed that block of conduction also takes place between the bundle of His and the bundle branches, and the bundle branches and the peripheral Purkinje tissue. It is suggested that block in these regions is due to the prolonged duration of the action potential rather than decremental conduction.

Moe *et al* (70) and Rosenblueth (71) postulated the existence of a dual atrioventricular transmission system involving two groups of fibers which differed with respect to duration of refractoriness and conduction velocity. They suggested that one system conducts at a higher velocity, and has a greater refractoriness than the other system. Their results were obtained from the measurement of the functional refractory period of atrioventricular transmission (FRP), defined by Krayer, Mandoki and Mendez (72) as being the minimal interval between two ventricular impulses propagated from the atrium.

The measurements of FRP were obtained from open chest dogs where the heart was driven electrically through stimulating electrodes attached to the atrium. Electrograms were recorded from both the atrium and ventricles. A premature

stimulus was applied to the atrium at shorter and shorter intervals after every sixth regular driving stimulus. As the extra stimulus is applied earlier and earlier in the driven cycle, the interval between the recorded ventricular responses shortens in direct proportion to the interval between the regular driving stimulus and the extra stimulus. A point is then reached where the interval between the two ventricular responses is not further decreased with decreases in the interval between the regular and premature stimuli. This minimal obtainable interval between the two ventricular responses propagated from the atrium is termed the functional refractory period of atrioventricular transmission.

In some experiments when the interval between the regular driving stimulus and the extra stimulus was shortened beyond the FRP, there appeared an abrupt or progressive increase in the delay between the two responses recorded from the ventricle. These authors attributed this abrupt change in the interval between the two recorded ventricular responses to a dual atrioventricular conducting system. They suggested that when the propagated premature impulse reaches the atrioventricular transmission system too soon after the preceding regular impulse, the rapidly conducting fibers are still refractory. Therefore the fibers which conduct more slowly but which have a shorter refractory period are activated and conduct the premature impulse to the ventricles.

Hoffman et al (73), however, disagree with the dual conduction hypothesis. They suggest that the abrupt increase in the interval between the regular driving stimulus and the premature stimulus recorded from the ventricles is associated with the absence of local electrical activity in some part of the peripheral Purkinje system. They studied the atrioventricular transmission of premature responses by recording the electrical activity directly from the atrium, AV-node, bundle of His, bundle branches, and peripheral Purkinje fibers of dogs. They found that premature activation of the bundle of His, bundle branches, or the peripheral Purkinje fibers resulted in slow or blocked conduction. Therefore a single impulse could be delayed at several sites in the atrioventricular transmission system. If this local delay is sufficient or if some Purkinje fibers completely fail to conduct, the pattern of ventricular activation would be changed. They suggest that as the interval between the premature impulse and the regular impulse is shortened, conduction is first delayed in the AV-node and secondly in the peripheral Purkinje fibers. The AV-nodal delay is postulated to result from decremental conduction, whereas the delay in the peripheral Purkinje fibers is concerned with premature activation.

An earlier study by Alanis et al (64) supports Hoffman's hypothesis of a delay occurring in more than one place in the atrioventricular transmission system. These workers

found that the FRP for the propagation between the AV-node and the bundle of His is longer than the FRP for propagation between the atrium and the AV-node. They postulated the existence of two functional discontinuities in the atrioventricular transmission system; one of them located between the AV-node and the bundle of His, and the other located either within the AV-node, or at the junction of the atrium and the AV-node.

B. CHEMISTRY

It has been reported (74) that when p-chloromercuribenzoic acid is dissolved in water the chloride and carboxyl groups ionize. The affinity of the hydroxide ion for the mercurial is greater than that of the chloride ion. Therefore the mercurial may exist as either the hydroxide, p-hydroxymercuribenzoic acid, or the chloride, p-chloromercuribenzoic acid, depending on the pH of the solution. Because of common usage this mercurial will be referred to as p-chloromercuribenzoic acid throughout this thesis.

C. STATEMENT OF PROBLEM

The purpose of this investigation is to determine the factors involved in the blockade of atrioventricular transmission caused by p-chloromercuribenzoic acid, and in the potentiation of this effect by methoxamine.

The question is whether these drugs cause blockade of

atrioventricular transmission by a direct effect on the conducting system of the heart, or by reflex mechanisms. Therefore the effect of these drugs on the blood pressure, heart rate, and electrocardiogram were analyzed to see if they were related to the blockade of atrioventricular transmission. Further investigation on the direct effect of these drugs on the atrioventricular conducting system was carried out by the measurement of the functional refractory period of atrioventricular transmission, and the conduction time of atrioventricular transmission.

SECTION II

METHODS

A. ANAESTHESIA

Seventy-six mongrel dogs unselected as to sex and weighing from 3.5 to 16.8 kg were anaesthetized with sodium pentobarbital (30 mg/kg) given intravenously. Anaesthesia was maintained when necessary by additional doses of 1-2 mg/kg.

Morphine sulphate (1 mg/kg) was administered subcutaneously to two dogs prior to the administration of chloralose (10 mg/kg). One animal was anaesthetized with a chloralose-urethane mixture (3 ml/kg) of the following composition:

Chloralose	1.9 gm
Urethane	19.0 gm
Water	90.0 ml

B. ARTIFICIAL RESPIRATION

All dogs were ventilated artificially by a Palmer Ideal respiration pump through a cannula inserted into the trachea. The dogs were supplied with room air at a rate of 18 cycles/min and a tidal volume of 20-30 ml/kg body weight.

C. SURGICAL PROCEDURES

1. Vagotomy

Bilateral mid-cervical vagotomy was done immediately after the induction of anaesthesia in all experiments in which the functional refractory period of atrioventricular trans-

mission was measured. This procedure was also done in a number of other experiments.

2. Stellate Ganglionectomy

Bilateral stellate ganglionectomy was done in 14 dogs. A skin incision was made in the axillary fold of the forelimb. A small incision was made in the intercostal muscle at the mid axillary level between the first and second ribs, producing a pneumothorax in all the animals. A mastoid retractor was inserted carefully to avoid puncturing the pleura. The surrounding tissue was dissected away and the stellate ganglion removed with as much of the chain as possible.

Sham stellate ganglionectomy was done in seven dogs. The same surgical procedure described above was carried out without touching the stellate ganglia or the sympathetic chain.

3. Thoracotomy and Pericardotomy

In experiments involving the measurement of the functional refractory period of atrioventricular transmission the sternum was exposed by electrocautery and cleaved in the midline over its entire length. The pericardium was incised and its edges sutured to the chest wall to form a pericardial cradle.

In all other experiments involving thoracotomy, electrocautery was employed to expose the intercostal muscles of the right thorax, and the pleural space was entered between

the 4th and 5th ribs by blunt dissection. A small incision was made in the pericardium over the right atrial appendage when the heart was to be paced electrically.

4. Procedures for Mechanical Control of the Systemic Blood Pressure

Mechanical elevation of the systemic blood pressure was done by reversibly occluding the thoracic aorta. A loose ligature was placed around the descending thoracic aorta and the ends were brought out through a stiff rubber tube. The vessel was reversibly occluded without displacement by compressing the rubber tubing against the aorta.

Lowering of the blood pressure was done by bleeding the animal through a polyethylene catheter of 2 mm internal diameter (Intramedic #260) inserted through the femoral artery and into the internal iliac artery. The pressure could be restored to control values by reinfusing the shed blood.

D. DRUGS

1. Injections of Drugs

Injections and infusions of all drugs except anaesthetics were made intravenously through a polyethylene catheter of 2 mm internal diameter (Intramedic #260) inserted through the femoral vein and into the common iliac vein. After each injection the catheter was flushed through with

0.9% saline. Infusions were administered with a Harvard infusion pump at the rate of 1 ml/kg/min.

2. Drugs Used

Sodium p-Chloromercuribenzoic Acid*

Solutions of p-chloromercuribenzoic acid (p-CMB) were prepared in 0.9% saline, usually in a concentration of 1 mg/ml. When higher concentrations were required 0.1 ml of 2% sodium hydroxide was added to facilitate solution. As control for the pH of the solution, injections were preceded by injections of 0.9% saline adjusted to the same pH with hydrochloric acid or sodium hydroxide.

Methoxamine

Solutions of dl-methoxamine hydrochloride were prepared in 0.9% saline in a concentration of 1 mg/ml.

Heparin

In experiments involving haemorrhage the animal was treated with heparin (3 mg/kg). Solutions of heparin were prepared in 0.9% saline in a concentration of 20 mg/ml.

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*Because of common usage, the abbreviation "p-CMB" has been retained in this thesis.

E. RECORDING TECHNIQUES

The systemic arterial blood pressure was recorded through a polyethylene cannula of 2 mm internal diameter (Intramedic #260) inserted into the right common carotid artery. The polyethylene cannula was connected to a Statham P23AA transducer. Lead II electrocardiograms were taken with subcutaneous needle electrodes. Both measurements were recorded on a Grass ink-writing polygraph at a paper speed of 25 mm/sec.

F. MEASUREMENT OF FUNCTIONAL REFRACTORY PERIOD (FRP) AND CONDUCTION TIME (CT) OF ATRIOVENTRICULAR TRANSMISSION

The functional refractory period of atrioventricular transmission was measured by a slight modification of the technique described by Krayer, Mandoki and Mendez (72). All experiments were done on dogs anaesthetized with sodium pentobarbital, bilaterally vagotomized, and artificially ventilated. The heart was driven at a rate of 20-30 beats/min greater than the initial heart rate and the driving rate remained constant throughout the individual experiment. A Tektronix stimulator provided rectangular impulses of 0.5 msec duration and double the threshold voltage. The driving stimulator was coupled to a counting device which triggered a second Tektronix stimulator so as to provide an additional pulse of the same characteristics after every eighth driving impulse. The interval between the eighth driving stimulus

and the interpolated test stimulus was varied systematically between the maximum possible at the driving rate and the minimum determined by the ability of the heart to respond. The outputs from both stimulators were led through interconnected isolation transformers to the single pair of stimulating electrodes attached to the tip of the right atrium. Atrial and ventricular electrograms were recorded simultaneously on a Grass polygraph with a paper speed of 50 mm/sec from two pairs of recording electrodes, one attached to the right atrial and the other the right ventricular epicardium.

The intervals between the response of the atrium to the eighth driving stimulus and the succeeding test stimulus were plotted against the intervals between the corresponding ventricular responses. The minimum interval between ventricular responses thus obtained gives the functional refractory period of atrioventricular transmission. A curve was fitted by eye to each series of readings. The lowest (horizontal) portion of this curve was taken as the functional refractory period.

Atrioventricular propagation time was taken as the delay between atrial and ventricular responses measured after steady-state conditions had been reached at the selected heart rate, i.e., taking the 4th beat after the interpolated beat. The recording and measuring technique used in the

determination of FRP and CT are illustrated in Figure 1.

G. ANALYSIS OF RECORDS

The intervals on the electrocardiograms and electrograms were determined by means of a measuring magnifier graduated to 0.1 mm. This allowed the intervals to be measured to $\pm$ 4 msec at a paper speed of 25 mm/sec, and $\pm$ 2 msec at a paper speed of 50 mm/sec. These measurements were made from standard reference points as follows. The length of P-R interval was measured from the beginning of the P wave to the beginning of the R wave on the electrocardiogram. The width of the QRS complex was taken as the distance from the beginning of the Q wave to the beginning of the S wave on the electrocardiogram. The mean blood pressure was calculated as (diastolic + 1/3 pulse pressure), taken from the blood pressure record. The heart rate was read directly from the electrocardiogram or electrogram.

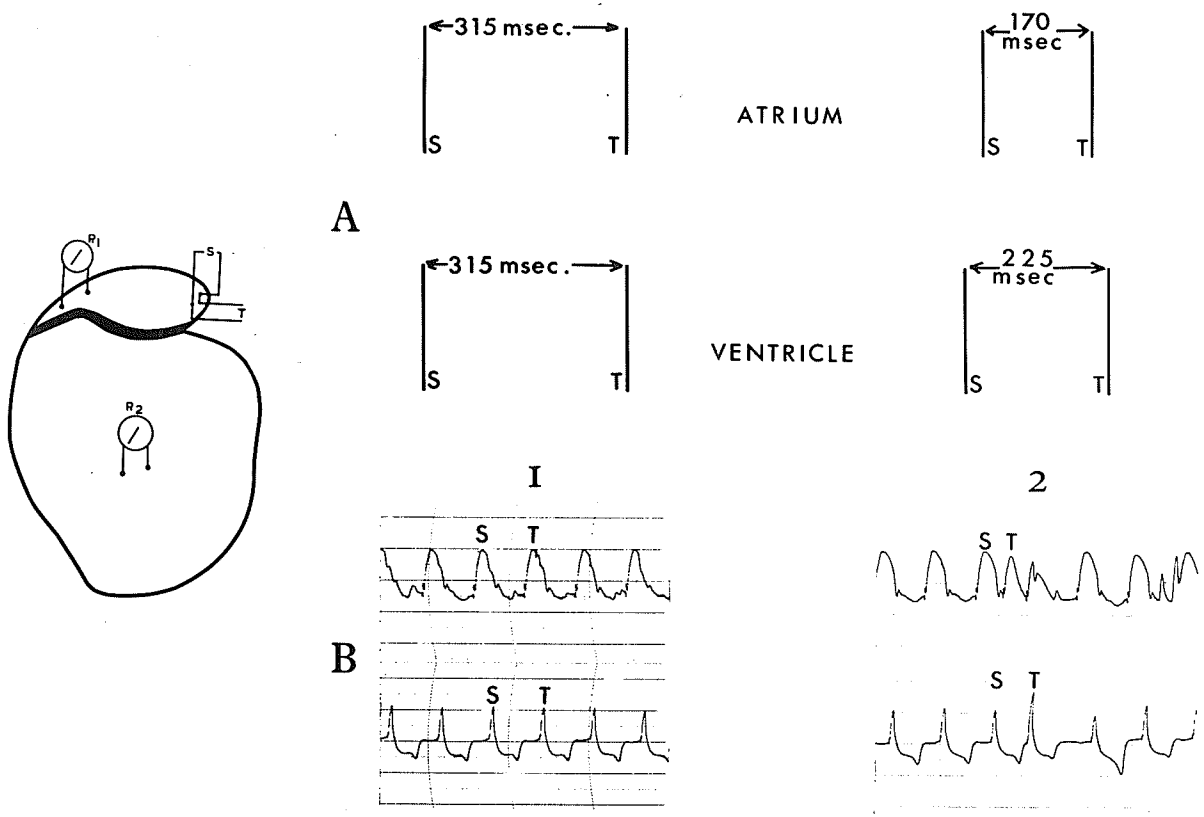

Figure 1. Left Diagramatic representation of the heart. R_1 & R_2 recording electrodes, S & T stimulating electrode. Right (A) Diagramatic representation of the intervals between the 8th regular stimulus (S) and the test stimulus (T) recorded from the atrial response (B-top tracing) and the ventricular response (B-bottom tracing). The record at the left (1) shows that at long intervals between the test and regular stimuli there is no delay in conduction between the atrium and ventricle. The record at the right (2) shows that at short intervals between the test and regular stimuli there is a delay in conduction between the atrium and ventricle.

SECTION III

ATRIOVENTRICULAR NODAL BLOCK DUE TO p-CHLOROMERCURIBENZOIC ACID AND THE EFFECT THEREON OF CHANGES IN CARDIAC INNERVATION AND OF PRETREATMENT WITH METHOXAMINE

A. INTRODUCTION

Farah et al (9) showed that p-CMB caused blockade of atrioventricular transmission. Ellis (75) confirmed that p-CMB caused varying degrees of AV-nodal blockade, and demonstrated that this toxicity was increased by methoxamine.

Preliminary investigation confirmed that p-CMB caused AV-nodal block, the incidence of which was increased by methoxamine. Further investigation presented here was therefore carried out to determine the factors involved in the blockade of atrioventricular transmission caused by p-CMB and in the potentiation of this effect by methoxamine.

B. RESULTS

1. Incidence of AV-Nodal Block

(a) Electrocardiographic Changes Produced by p-CMB

The administration of increasing doses of p-CMB (20-80 mg/kg) caused changes in the electrocardiogram similar to those observed by Farah (9) and illustrated in Figure 2. The first changes in the electrocardiogram were an elevated T wave and a depressed ST segment followed by an increase in the P-R interval. There were no changes in the QRS complex after the administration of p-CMB (see section 4 (c) below).

The above changes in the electrocardiogram were followed by AV-nodal block as evidenced by atrial depolarization

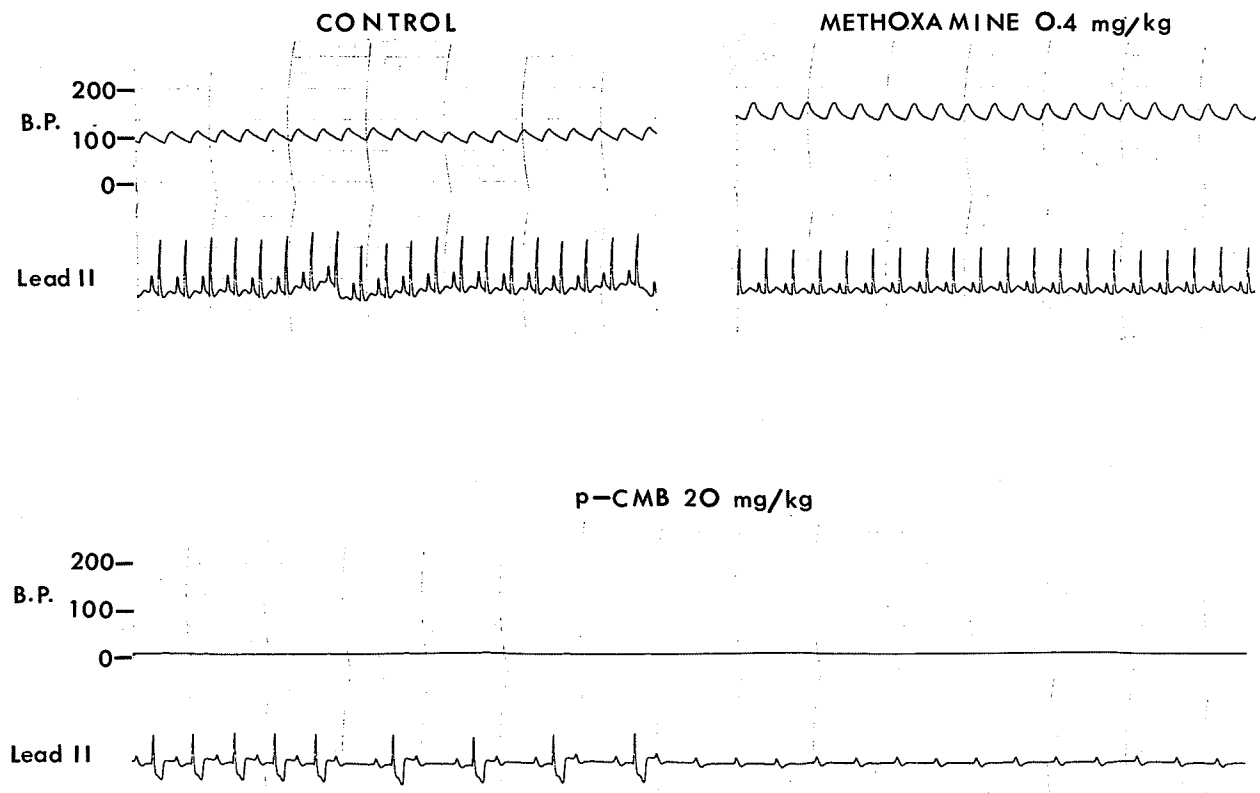

Figure 2. A typical record showing the toxic effects of p-CMB (20 mg/kg) on the electrocardiogram of a vagotomized dog pretreated with methoxamine.

(P waves) without the accompanying ventricular depolarization (QRS complex). The degree of AV-nodal block varied from 2-1 to complete block with a duration of 1-1.5 minutes. When complete AV-nodal block occurred the appearance of ventricular escape beats was rare. Complete AV-nodal block was observed in some animals for as long as 1.5 minutes without any ventricular escape beats. Blockade was terminated by either recovery to sinus rhythm or asystole and death. Ventricular fibrillation never occurred.

(b) The Incidence of AV-Nodal Block in Response to p-CMB

The incidence of AV-nodal block was a function of the dose of p-CMB. Eight dogs with intact vagal and sympathetic innervation of the heart were given varying initial doses of p-CMB; five received 20 mg/kg, two 30 mg/kg, and one 40 mg/kg. Five of the eleven dogs received additional doses of p-CMB (10-40 mg/kg).

The relationship between the cumulative dose of p-CMB and the cumulative incidence of AV-nodal block in these eleven dogs is illustrated in Figure 3 (dashed line). The percent of dogs developing AV-nodal block is compared to the dose of p-CMB. The assumption was made that those animals developing AV-nodal block after the initial dose of p-CMB would also have developed AV-nodal block at higher doses.

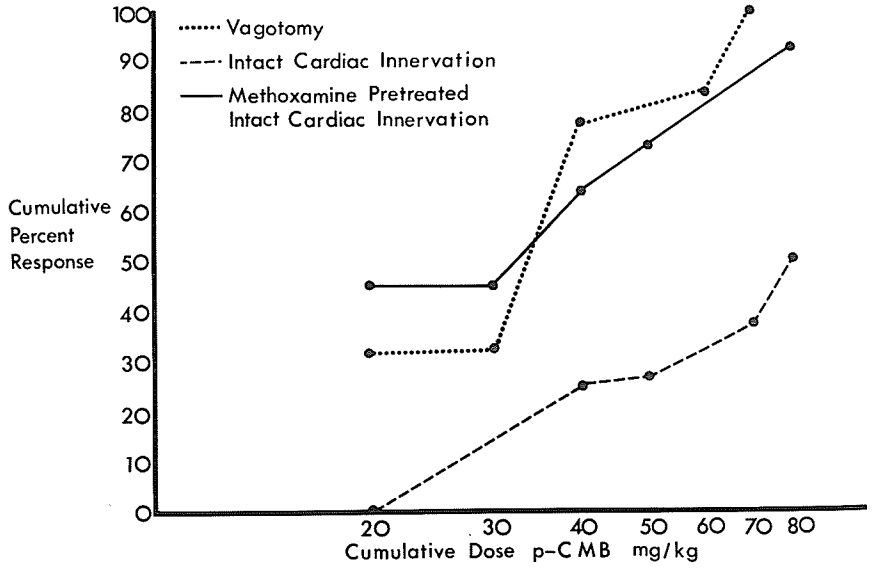

Figure 3. Dose-response curves of AV-nodal block due to p-CMB. Six vagotomized dogs ..... Eight dogs with intact cardiac innervation ------. Eleven dogs with intact cardiac innervation pre-treated with methoxamine (0.4 or 1.6 mg/kg) _____.

An additional three dogs received a constant infusion of p-CMB (1.0 mg/kg/min). Two of these dogs developed AV-nodal block; one at a total dose of 60 mg/kg and the other at a total dose of 80 mg/kg. The third animal did not develop AV-nodal block after a total dose of 80 mg/kg p-CMB.

Varying doses of p-CMB were administered to six vagotomized dogs to see if vagotomy would alter the response. Three of these dogs received initial doses of 20 mg/kg. Two of these three animals did not develop AV-nodal block and were given additional doses of p-CMB (10-30 mg/kg). Three additional animals received initial doses of 40 mg/kg. The dose response curve for all six animals is illustrated in Figure 3 (dotted line).

There was a higher incidence of AV-nodal block in the vagotomized dogs than in the dogs with intact cardiac innervation. However this difference may have been due to the different initial doses of p-CMB in the two groups of animals. Half of the vagotomized animals received initial doses of 40 mg/kg while only one of the intact animals received so large an initial dose. The apparently increased incidence of AV-nodal block at 20 mg/kg is based mainly on the response of one of three vagotomized animals, thus making this dose-response curve not entirely reliable.

(c) The Effect of Pretreatment with Methoxamine on the Incidence of AV-Nodal Block Produced by p-CMB

Eleven dogs with intact cardiac innervation were given methoxamine three minutes prior to the initial dose of p-CMB (20 mg/kg). The dose of methoxamine was 0.4 mg/kg in eight animals; three animals received 1.6 mg/kg. Six of the eleven dogs that did not develop AV-nodal block in response to the initial dose of p-CMB were given additional doses of 10-60 mg/kg. The dose response curve for these animals is shown in Figure 3 (solid line). It is apparent from Figure 3 that methoxamine increases the incidence of AV-nodal block caused by p-CMB.

(d) The Effect of Changes in Cardiac Innervation on the Increase in Incidence of AV-Nodal Block After Methoxamine Pretreatment

Experiments were carried out to determine whether the increased incidence of AV-nodal block caused by methoxamine pretreatment was due to a reflex response to the high dose of this pressor agent. The reflex increase in vagal tone and decrease in sympathetic tone to the heart in response to the pressor effect of methoxamine are well known phenomena, and have indeed been used in the treatment of sinus tachycardias (76,77). These reflex effects would also depress conduction through the AV-node, and might therefore account for the in-

creased incidence of AV-nodal block caused by p-CMB after methoxamine.

The cardiac innervation of twenty-eight dogs was altered in four different ways in order to elucidate the role of nervous influences on methoxamine potentiation of p-CMB induced AV-nodal block. The following procedures were undertaken to either eliminate partially or completely the above reflex effects.

(i) Bilateral vagotomy was done in four dogs, and three dogs were treated with atropine sulphate (1.0 mg/kg).

(ii) Bilateral stellate ganglionectomy was done on six dogs with intact vagi.

(iii) Bilateral stellate ganglionectomy plus vagotomy was done on eight dogs.

(iv) Sham stellate ganglionectomy plus bilateral vagotomy was done on seven dogs.

All the animals were given the same dose of methoxamine (0.4 mg/kg), and an initial dose of p-CMB (20 mg/kg). A summary of the responses of these animals is illustrated in Figure 4 (bars D,E,F,G). Also included in this figure are the responses to this dose of p-CMB of intact and vagotomized dogs not pretreated with methoxamine (bars A & B), and the pretreated dogs with intact cardiac innervation (bar C). It is obvious that neither vagotomy nor stellate ganglionectomy alone decreased the potentiation of p-CMB AV-nodal block by

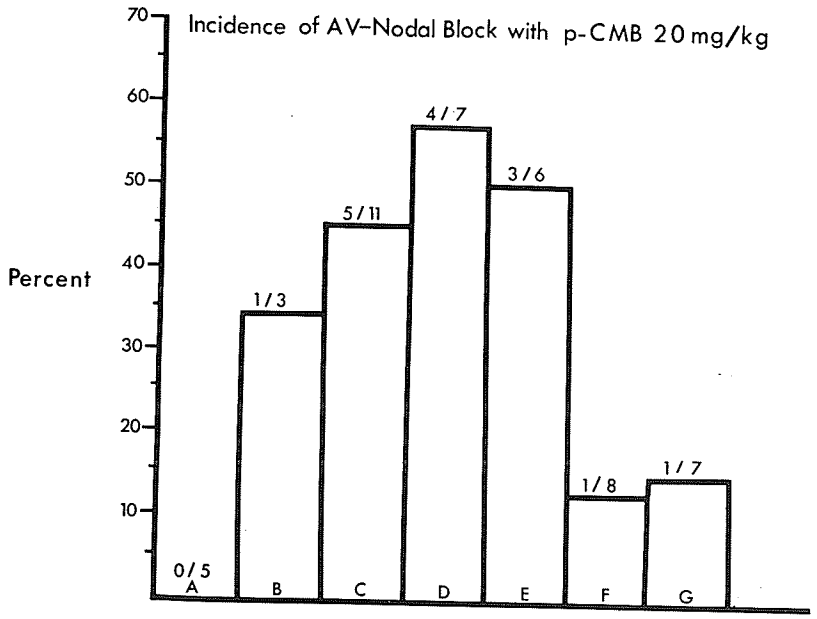

Figure 4. The incidence of AV-nodal block in response to 20 mg/kg p-CMB. The bars show the percent response with the actual incidence indicated above each bar. A. Dogs with intact cardiac innervation. B. Vagotomized dogs. C. Dogs with intact cardiac innervation pretreated with methoxamine. D. Vagotomized dogs pretreated with methoxamine. E. Stellate ganglionectomized dogs pretreated with methoxamine. F. Vagotomized, stellate ganglionectomized dogs pretreated with methoxamine. G. Vagotomized, sham stellate ganglionectomized dogs pretreated with methoxamine.

methoxamine (bars D & E). A combination of these procedures (bar F) prevented the methoxamine potentiation. However, the physiological significance of these results are negated by the observation that sham stellate ganglionectomy combined with vagotomy was equally effective in preventing the methoxamine potentiation (bar G).

Most of the animals which did not develop AV-nodal block in response to the initial dose of p-CMB (20 mg/kg) were given additional doses of 10-60 mg/kg p-CMB. This additional treatment was given as follows:

Group (i) : 3 of 4 dogs not developing AV-nodal block

Group (ii) : 2 of 3 dogs not developing AV-nodal block

Group (iii): 5 to 7 dogs not developing AV-nodal block

Group (iv) : 6 of 6 dogs not developing AV-nodal block

As expected, additional doses of p-CMB increased the incidence of AV-nodal block. The resulting dose-response curves are shown in Figures 5 and 6, each which also shows the dose response curve for the dogs with intact cardiac innervation. The curves for the vagotomized dogs (group i) and the stellate ganglionectomized dogs (group ii) are substantially identical with control curves for the dogs with intact cardiac innervation. However the curve showing the incidence of AV-nodal block in the sham stellate ganglionectomized, vagotomized dogs appears to be somewhat flatter. It may be concluded, however, that the state of the cardiac innervation has little or no effect on the potentiation of AV-nodal block.

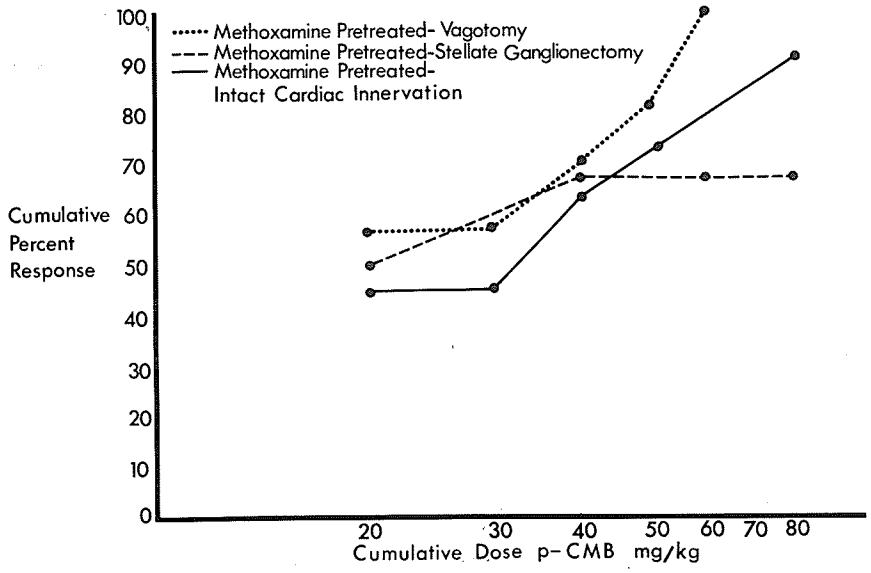

Figure 5. Dose-response curves of AV-nodal block due to p-CMB. Seven vagotomized dogs pretreated with methoxamine (0.4 mg/kg)..... Six stellate ganglionectomized dogs pretreated with methoxamine (0.4 mg/kg) ------. Eleven dogs with intact cardiac innervation pretreated with methoxamine (0.4 or 1.6 mg/kg) _____.

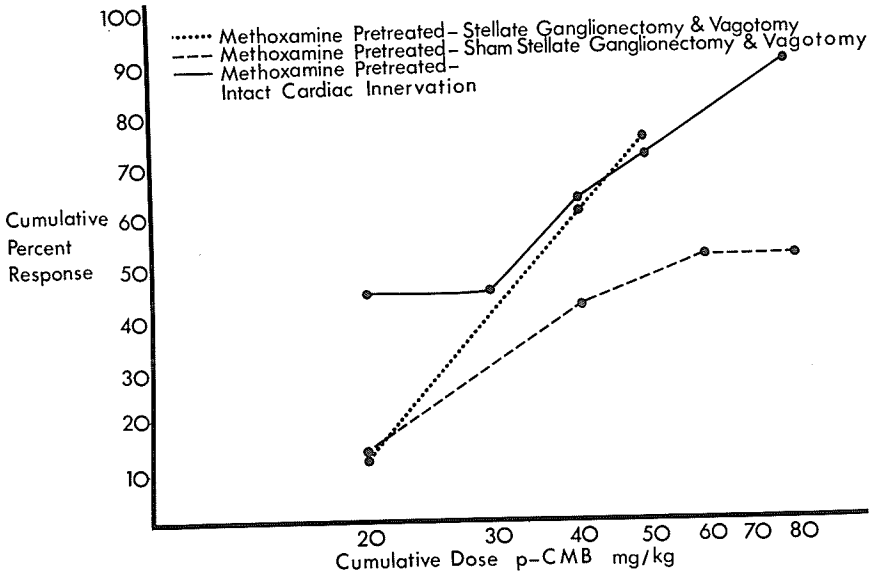

Figure 6. Dose-response curves of AV-nodal block due to p-CMB. Eight stellate ganglionectomized, vagotomized dogs pretreated with methoxamine (0.4 mg/kg) ..... Seven sham stellate ganglionectomized, vagotomized dogs pretreated with methoxamine (0.4 mg/kg) ------. Eleven dogs with intact cardiac innervation pretreated with methoxamine (0.4 or 1.6 mg/kg) _____.

2. Analysis of the Influence of Blood Pressure on the Incidence of AV-Nodal Block

The previous results indicate that methoxamine increased the incidence of AV-nodal block caused by p-CMB, and that this effect of methoxamine was not due to reflex mechanisms altering sympathetic or vagal tone to the heart. An increase in the blood pressure per se has been shown to decrease atrioventricular conduction (78). It is possible, therefore, that methoxamine exerts its potentiating effect through a direct effect of its pressor activity on the heart. Statistical analysis was done to determine whether the increase in blood pressure caused by methoxamine or the fall in blood pressure caused by p-CMB was related to the incidence of AV-nodal block. Both the amount of change and the level of the blood pressure after methoxamine or p-CMB were analyzed. The increase in blood pressure caused by methoxamine was taken as the maximal blood pressure obtained, and the fall in blood pressure caused by p-CMB was taken as the minimum blood pressure obtained before AV-nodal block. All blood pressures reported in this section are mean pressures (diastolic + 1/3 pulse pressure) except where otherwise indicated. All average blood pressures are given with their standard errors, and are compared by Student's t-test.

(a) The Role of the Rise in Blood Pressure in Response to Methoxamine in the Increased Incidence of AV-Nodal Block

The administration of methoxamine (0.4 and 1.6 mg/kg) prior to p-CMB caused a marked increase in the blood pressures of thirty-nine dogs. An analysis of this rise in blood pressure was undertaken to see if it was correlated with the incidence of AV-nodal block caused by subsequent administration of p-CMB (20 mg/kg). Table I summarizes the data obtained from these thirty-nine dogs as a whole group, and from the same thirty-nine dogs divided into five subgroups (2-6) according to their cardiac innervations. Fourteen of the thirty-nine dogs developed AV-nodal block in response to p-CMB (20 mg/kg). However the rise in blood pressure after methoxamine was not significantly greater in the fourteen animals developing AV-nodal block than in the twenty-five dogs not developing AV-nodal block. This lack of significance held true if the thirty-nine dogs were considered as a group or if they were considered according to the state of their cardiac innervation.

Similar analysis was made comparing the effect of the absolute level of blood pressure after methoxamine on the incidence of AV-nodal block caused by subsequent administration of 20 mg/kg p-CMB. Table II shows that the level of the blood pressure was not significantly greater in the fourteen dogs developing AV-nodal block than in the twenty-five dogs

TABLE I

A COMPARISON BETWEEN THE INCREASES IN BLOOD PRESSURE AFTER METHOXAMINE IN DOGS DEVELOPING AV-NODAL BLOCK AFTER p-CMB (20 mg/kg) AND THOSE NOT DEVELOPING AV-NODAL BLOCK AFTER p-CMB (20 mg/kg)

Group	Incidence of AV-Nodal Block	AVERAGE RISE IN BLOOD PRESSURE AFTER METHOXAMINE				
		Dogs Developing AV-Nodal Block after p-CMB	S.E.	Dogs not developing AV-Nodal Block after p-CMB	S.E.	P
1) All dogs pretreated with Methoxamine regardless of the cardiac innervation	14/39	69.2	7.1	61.8	5.0	> 0.3
2) Intact Innervation	5/11	68.6	7.8	62.3	11.32	> 0.6
3) Vagotomy	4/7	76.0	24.8	59.6	20.8	> 0.6
4) Stellate Ganglionectomy	3/6	64.3	9.7	64.3	7.3	*
5) Vagotomy + Stellate Ganglionectomy	1/8	67.0	*	59.2	9.9	*
6) Vagotomy + Sham Stellate Ganglionectomy	1/7	63.0	*	64.0	12.2	*

* not calculated because of distribution of effect

TABLE II

A COMPARISON BETWEEN THE MAXIMAL BLOOD PRESSURE OBTAINED AFTER METHOXAMINE IN DOGS DEVELOPING AV-NODAL BLOCK AFTER p-CMB (20 mg/kg) AND THOSE NOT DEVELOPING AV-NODAL BLOCK AFTER p-CMB (20 mg/kg)

		AVERAGE LEVELS OF MAXIMUM BLOOD PRESSURE AFTER METHOXAMINE				
Group	Incidence of AV-Nodal Block	Dogs Developing AV-Nodal Block After p-CMB	S.E.	Dogs not Developing AV-Nodal Block after p-CMB	S.E.	P
1) All dogs pretreated with Methoxamine regardless of the cardiac innervation	14/39	175.8	7.2	154.7	6.8	> 0.05
2) Intact Innervation	5/11	195.2	7.0	166.1	12.3	> 0.05
3) Vagotomy	4/7	176.7	16.3	155.3	49.1	> 0.6
4) Stellate Ganglionectomy	3/6	155.3	9.7	165.3	9.2	> 0.4
5) Vagotomy + Stellate Ganglionectomy	1/8	140.0	*	137.4	10.8	*
6) Vagotomy + Sham Stellate Ganglionectomy	1/7	173.0	*	157.8	5.3	*

* Not calculated because of distribution of effect

not developing AV-nodal block.

The above comparisons were also made using systolic instead of mean blood pressures (Table III). Neither the rise, nor the maximum level of blood pressure were correlated with the incidence of AV-nodal block.

(b) The Effect of p-CMB on the Blood Pressure

p-CMB causes a fall in the blood pressure. An analysis was done to determine whether the degree of this fall, or the absolute levels reached, were related to the incidence of AV-nodal block. Although it had been shown in the previous results (section (a) above) that neither the rise nor the absolute level of the blood pressure after methoxamine was correlated with the incidence of AV-nodal block, analysis was also done to determine whether methoxamine influenced either the fall in blood pressure or the minimum level reached after p-CMB.

The administration of p-CMB (20-80 mg/kg) caused a rapid fall in the blood pressure of fifty-three dogs, thirty-nine of which were pretreated with methoxamine (0.4 or 1.6 mg/kg). The relationship between the magnitude of this fall in blood pressure and the dose of p-CMB is illustrated in the dose-response curves shown in Figure 7. The average blood pressures were calculated as percent of initial blood pressure, and the dose of p-CMB is a cumulative dose. The magnitude of the fall in blood pressure increased with the dose of p-CMB

TABLE III

A COMPARISON BETWEEN THE SYSTOLIC BLOOD PRESSURE AFTER METHOXAMINE IN DOGS DEVELOPING AV-NODAL BLOCK AFTER p-CMB (20 mg/kg) AND THOSE DOGS NOT DEVELOPING AV-NODAL BLOCK AFTER p-CMB (20 mg/kg)

	Average Systolic Blood Pressure with Standard Errors		
	14 Dogs Developing AV-Nodal Block after p-CMB (20 mg/kg)	25 Dogs Not Developing AV-Nodal Block After p-CMB (20 mg/kg)	P
Rise in Systolic Blood Pressure after Methoxamine	74.2 ± 8.1	72.0 ± 5.6	> 0.8
Level of the Systolic Blood Pressure After Methoxamine	202.8 ± 7.9	184.4 ± 7.4	< 0.05

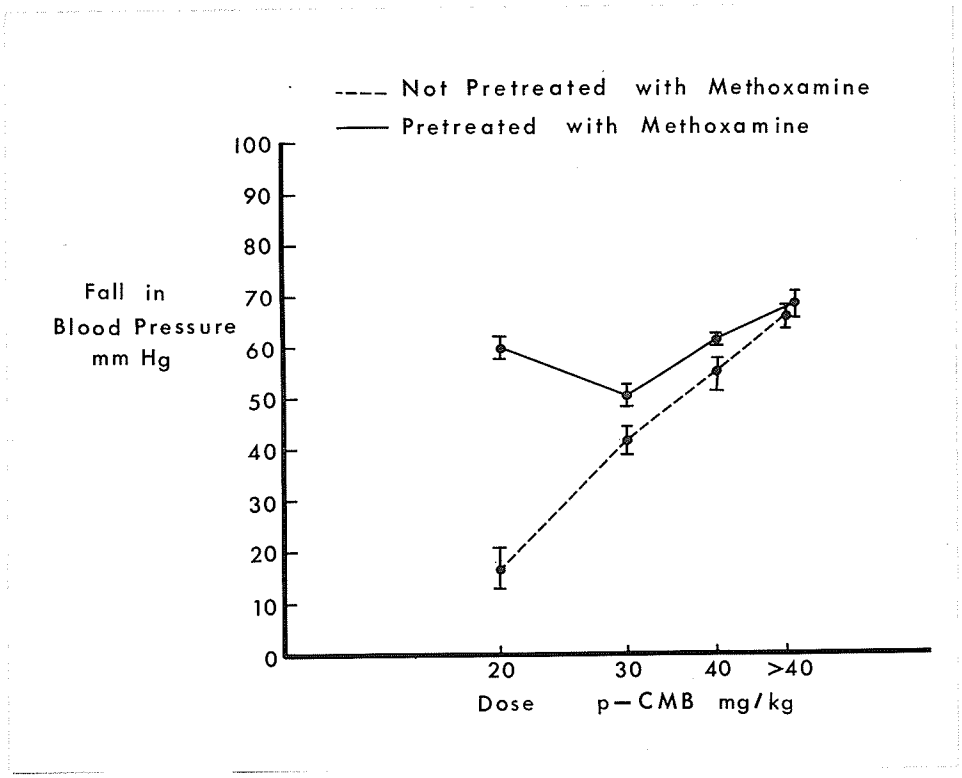

Figure 7. Dose-response curves of the fall in blood pressure caused by p-CMB calculated as % of initial. The average blood pressures are given with their standard errors. Fourteen dogs not pretreated with methoxamine ------. Thirty-nine dogs pretreated with methoxamine (0.4 or 1.6 mg/kg) _____.

in the group of animals not pretreated with methoxamine. However the methoxamine pretreated animals showed very little change in the magnitude of the fall in blood pressure with increasing doses of p-CMB. The methoxamine-pretreated animals demonstrated a significantly greater fall in the blood pressure in response to the standard initial dose of 20 mg/kg p-CMB than did the animals not treated with methoxamine ($P < 0.01$ - Table IV).

The relationship between the cumulative dose of p-CMB and the absolute level of the blood pressure obtained in response to p-CMB is illustrated in Figure 8. In both groups of animals the level of the blood pressure obtained after 30 mg/kg p-CMB was no less than that obtained after 20 mg/kg. However, 40 mg/kg caused an abrupt change to a lower level of blood pressure in the group of animals not treated with methoxamine. Doses larger than 40 mg/kg caused a decrease in the level of the blood pressure in both groups of animals.

Despite their higher initial blood pressures the methoxamine-pretreated animals had a lower blood pressure after 20 mg/kg p-CMB than did the animals not treated with methoxamine. However, this difference did not approach statistical significance ($P > 0.1$ - Table IV). Approximately the same level of blood pressure was obtained in both groups of animals in response to doses of 40 mg/kg or more of p-CMB.

The above results indicate that the magnitude of the

TABLE IV

A COMPARISON BETWEEN THE AVERAGE BLOOD PRESSURE AFTER p-CMB (20 mg/kg) IN THOSE DOGS PRETREATED WITH METHOXAMINE AND THOSE DOGS NOT PRETREATED WITH METHOXAMINE

	Average Blood Pressure with Standard Errors		
	39 Dogs Pretreated with Methoxamine	8 Dogs Not Pretreated With Methoxamine	P
Fall in Mean Blood Pressure After p-CMB (20 mg/kg)	97.8 ± 8.3	36.7 ± 14.1	< 0.01
Level of the mean Blood Pressure After p-CMB (20 mg/kg)	66.9 ± 6.6	89.5 ± 11.9	> 0.1

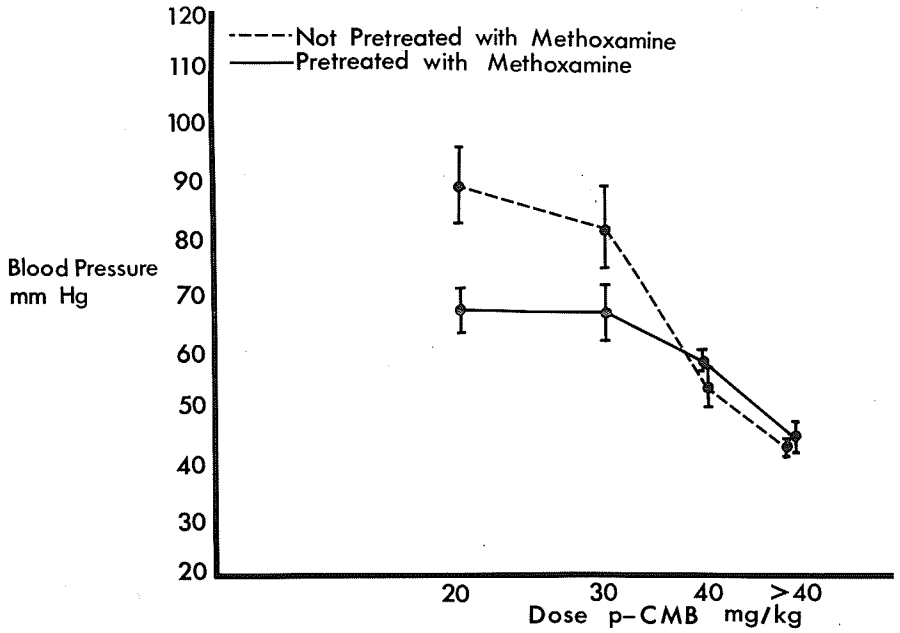

Figure 8. Dose-response curves of the level of the blood pressure obtained after p-CMB. The average blood pressures are given with their standard errors. Fourteen dogs not pretreated with methoxamine ------. Thirty-nine dogs pretreated with methoxamine (0.4 or 1.6 mg/kg) _____.

fall in blood pressure in response to p-CMB in the methoxamine-pretreated animals did not increase with the dose. This is reflected by the same level of blood pressure being obtained with either 20 or 30 mg/kg p-CMB. Similar results were also obtained with the incidence of AV-nodal block and the dose of p-CMB (section 1), or the P-R interval (section 4). The dose-response curves in Figures 3, 4 and 11, page 66, show that the incidence of AV-nodal block was the same in response to 20 or 30 mg/kg p-CMB. An explanation for these unusual observations may be that the animals which did not respond to 20 mg/kg p-CMB showed considerable resistance to the effects of p-CMB, and therefore only responded to higher doses of p-CMB (40-80 mg/kg).

(c) <u>The Effect of the Fall and Level of the Blood Pressure After p-CMB on the Incidence of AV-Nodal Block</u>

The above results indicated that p-CMB had greater effect on the blood pressure in the methoxamine-pretreated animals than in the animals not treated with methoxamine. As this might be a possible mechanism of methoxamine potentiation, the data were analyzed further in an attempt to relate the decreases in blood pressure with the incidence of AV-nodal block.

The relationship between the magnitude of the fall in

blood pressure after p-CMB (20 - 80 mg/kg) and the incidence of AV-nodal block is illustrated in Figure 9. Fifty-three dogs were divided into four groups: (a) Those dogs receiving p-CMB (20 mg/kg) without prior administration of methoxamine. (b) Those dogs receiving p-CMB (regardless of the dose) without prior administration of methoxamine. (c) Those dogs pretreated with methoxamine (0.4 or 1.6 mg/kg) and receiving p-CMB (20 mg/kg). (d) Those dogs pretreated with methoxamine (0.4 or 1.6 mg/kg) and receiving p-CMB (regardless of the dose). The incidence of AV-nodal block in these four groups of animals is expressed as a percent, since both those animals developing AV-nodal block and those animals not developing AV-nodal block are included. The ranges of blood pressure were selected arbitrarily and compared with the incidence of AV-nodal block in each of the above four groups of dogs. A greater fall in the blood pressure was associated with an increased incidence of AV-nodal block in response to p-CMB in all four groups of dogs.

The same comparison as above was made with the level of the blood pressure after p-CMB (20-80 mg/kg), and the incidence of AV-nodal block (Figure 10). A higher incidence of AV-nodal block in response to p-CMB was also associated with a lower blood pressure in all four groups of dogs.

Further analysis was undertaken to see if there was statistical evidence for the above relationship between the

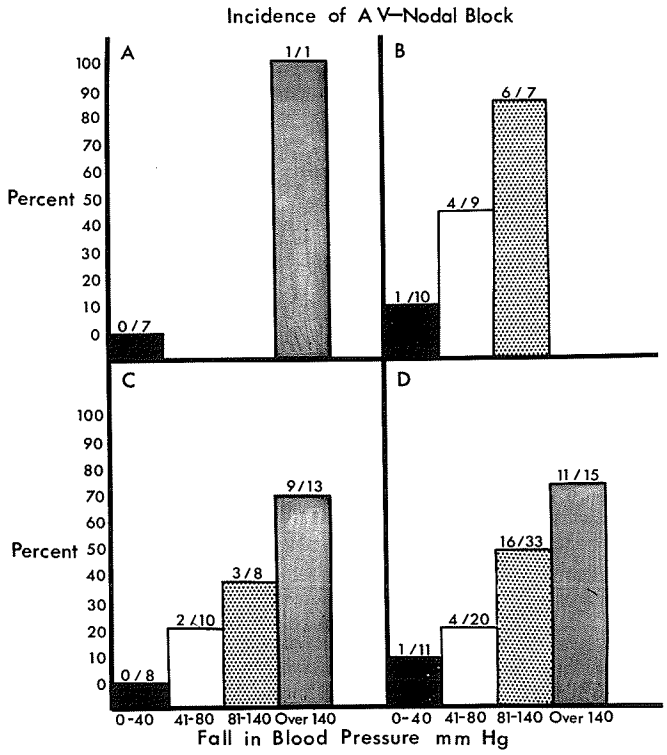

Figure 9. The relationship of the fall in mean blood pressure caused by p-CMB and the incidence of AV-nodal block. The bars represent dogs grouped according to their blood pressures just before block. The figures over the bars show the actual incidence of AV-nodal block. The ordinates indicate the incidence of AV-nodal block as a percent. A. Those dogs receiving p-CMB (20 mg/kg) without prior administration of methoxamine. B. Those dogs receiving p-CMB (regardless of the dose) without prior administration of methoxamine. C. Those dogs pretreated with methoxamine (0.4 or 1.6 mg/kg) and receiving p-CMB (20 mg/kg). D. Those dogs pretreated with methoxamine (0.4 or 1.6 mg/kg) and receiving p-CMB (regardless of the dose).

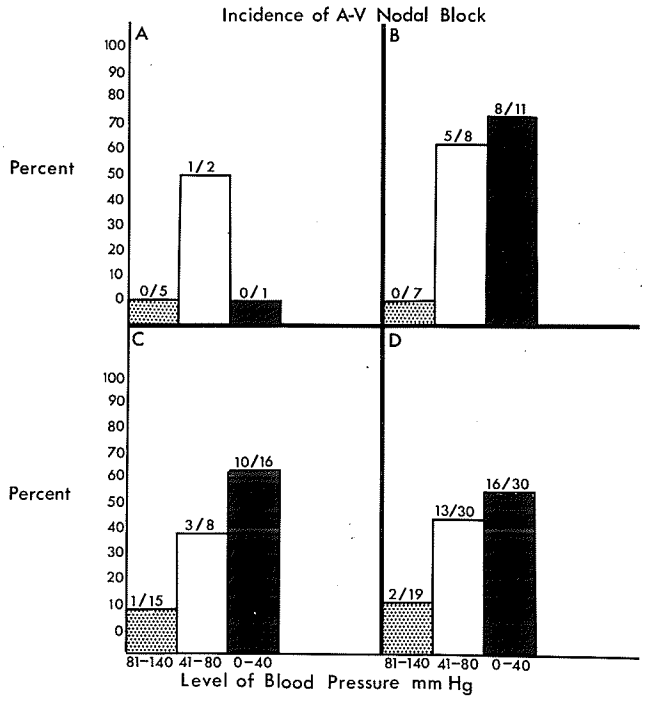

Figure 10. The relationship of the level of mean blood pressure after p-CMB and the incidence of AV-nodal block. The bars represent dogs grouped according to their blood pressures just before block. The figures over the bars show the actual incidence of AV-nodal block. The ordinates indicate the incidence of AV-nodal block as a percent. A. Those dogs receiving p-CMB (20 mg/kg) without prior administration of methoxamine. B. Those dogs receiving p-CMB (regardless of the dose) without prior administration of methoxamine. C. Those dogs pretreated with methoxamine (0.4 or 1.6 mg/kg) and receiving p-CMB (20 mg/kg). D. Those dogs pretreated with methoxamine (0.4 or 1.6 mg/kg) and receiving p-CMB (regardless of the dose).

incidence of AV-nodal block and either the fall in blood pressure or the level of the blood pressure obtained in response to p-CMB. A comparison of the blood pressure response to p-CMB (20 mg/kg) was made between those animals developing AV-nodal block and those animals not developing AV-nodal block in response to 20 mg/kg p-CMB. The average blood pressures used in this comparison were obtained from forty-seven dogs, thirty-nine of which were pretreated with methoxamine. The results of this comparison are summarized in Tables V and VI.

One of eight dogs not pretreated with methoxamine developed AV-nodal block in response to p-CMB (20 mg/kg). This dog demonstrated a greater fall to a lower level of blood pressure than the remaining seven dogs (Tables V (3), and VI (3)).

Fourteen of the thirty-nine methoxamine-pretreated dogs developed AV-nodal block in response to p-CMB (20 mg/kg). These fourteen dogs showed a significantly greater fall to a lower level of blood pressure $P < 0.001$, and $P < 0.001$ respectively (Tables V (4), and VI (4)).

This clear cut difference in the blood pressure response to p-CMB (20 mg/kg) between those animals developing AV-nodal block and those animals not developing AV-nodal block in the whole group of thirty-nine methoxamine-pretreated dogs did not hold true if these dogs were divided into sub

TABLE V

A COMPARISON BETWEEN THE FALL IN BLOOD PRESSURE AFTER p-CMB (20 mg/kg) IN DOGS DEVELOPING AV-NODAL BLOCK AND THOSE NOT DEVELOPING AV-NODAL BLOCK

Group	Incidence of AV-Nodal Block	Average Fall in Blood Pressure After p-CMB (20 mg/kg)				
		Dogs developing AV-Nodal Block after p-CMB	S.E.	Dogs not developing AV-Nodal Block after p-CMB	S.E.	P
Not pretreated with Methoxamine						
1) Intact Innervation	0/5		*	23.4	6.5	*
2) Vagotomy	1/3	130.0	*	23.5	10.6	*
3) Combination of Intact and vagotomized groups	1/8	130.0	*	23.4	1.5	*
Pretreated with Methoxamine						
4) All Dogs Pretreated with Methoxamine regardless the cardiac innervation	14/39	137.2	9.7	75.8	9.6	< 0.001
5) Intact Innervation	5/11	168.8	4.1	83.6	21.6	< 0.001
6) Vagotomy	4/7	116.0	16.4	83.0	35.4	> 0.3
7) Stellate Ganglionectomy	3/6	118.3	23.7	132.0	10.2	> 0.6
8) Vagotomy + Stellate Ganglionectomy	1/8	97.0	*	57.5	12.5	*
9) Vagotomy + Sham Stellate Ganglionectomy	1/7	160.0	*	57.8	20.3	*

* Not calculated because of distribution of effect

TABLE VI

A COMPARISON BETWEEN THE LEVEL OF BLOOD PRESSURE OBTAINED AFTER p-CMB (20 mg/kg) IN DOGS DEVELOPING AV-NODAL BLOCK AND THOSE NOT DEVELOPING AV-NODAL BLOCK

Group	Incidence of AV-Nodal Block	AVERAGE LEVEL OF BLOOD PRESSURE AFTER P-CMB (20 mg/kg)				
		Dogs developing AV-Nodal Block After p-CMB	S.E.	Dogs not developing on AV-Nodal Block After p-CMB	S.E.	P
Not pretreated with Methoxamine						
1) Intact Innervation	0/5		*	90.6	13.3	*
2) Vagotomy	1/3	50.0	*	106.5	33.8	*
3) Combination of Intact and vagotomized groups	1/8	50.0	*	95.1	11.7	*
Pretreated with Methoxamine						
4) All dogs Pretreated with Methoxamine regardless of the Cardiac Innervation	14/39	31.2	5.2	82.8	7.4	< 0.001
5) Intact Innervation	5/11	26.4	5.0	82.5	12.6	< 0.001
6) Vagotomy	4/7	60.5	27.7	72.3	16.4	> 0.7
7) Stellate Ganglionectomy	3/6	37.0	22.3	33.3	10.5	> 0.8
8) Vagotomy + Stellate Ganglionectomy	1/8	43.0	*	79.8	15.1	*
9) Vagotomy + Sham Stellate Ganglionectomy	1/7	13.0	*	116.0	6.2	*

* Not calculated because of distribution of effect.

groups (Tables V (5-9), and VI (5-9)) according to their cardiac innervation.

Five of eleven dogs with intact cardiac innervation developed AV-nodal block in response to p-CMB (20 mg/kg). These five dogs demonstrated a significantly greater fall to a lower level of blood pressure $P < 0.001$ and $P < 0.001$ respectively (Tables V (5), and VI (5)).

Four of seven vagotomized dogs developed AV-nodal block in response to p-CMB (20 mg/kg). The fall and the level of blood pressure were not significantly greater in these four dogs $P > 0.3$ and $P > 0.7$ respectively. (Tables V (6), and VI (6)).

Three of six stellate ganglionectomized dogs developed AV-nodal block in response to p-CMB (20 mg/kg). The three dogs not developing AV-nodal block had a greater fall to a lower level of blood pressure; however, this was not significant $P > 0.6$ and $P > 0.8$ respectively (Tables V (7), and VI (7)).

One of eight vagotomized and stellate ganglionectomized dogs developed AV-nodal block in response to p-CMB (20 mg/kg). This one dog demonstrated a greater fall to a lower level of blood pressure (Tables V (8), and VI (8)).

One of seven vagotomized and sham stellate ganglionectomized dogs developed AV-nodal block in response to p-CMB (20 mg/kg). This one dog showed a greater fall to a

lower level of blood pressure than the other seven dogs (Tables V (9), and VI (9)).

All the changes in blood pressure reported above were calculated as absolute change. Therefore an additional analysis was carried out which showed that there was no difference in the significance of the changes in blood pressure whether they were calculated as absolute or as percent of the initial level of blood pressure.

These results indicate that the blood pressure response to p-CMB (20 mg/kg) is related to the incidence of AV-nodal block. However the blood pressure is not the only factor involved. For example if the cardiac innervation is changed the blood pressure is no longer clearly a determinant factor in the production of AV-nodal block.

3. Analysis of the Role of Changes in Heart Rate on the Incidence of AV-Nodal Block

Changes in the heart rate can influence atrioventricular transmission. If the sinus rate is greater than the refractory period of the AV-node, atrioventricular conduction will be blocked. Some indication as to the mechanism of methoxamine potentiation would be apparent if the changes in heart rate produced by methoxamine or p-CMB were correlated with the incidence of AV-nodal block. Therefore, a statistical analysis was done to determine if there was a relationship between changes in heart rate and the incidence of AV-nodal block.

The changes in heart rate produced by methoxamine were taken as the maximum change before the administration of p-CMB. The changes in heart rate produced by p-CMB were taken as the maximum before AV-nodal block. The changes in heart rate were compared by Student's t-test, and by Student's t-test for paired data. All means are given with their standard errors.

(a) The Effect of Methoxamine on the Heart Rate

The changes in heart rate after methoxamine were analyzed statistically in thirty-nine dogs with various cardiac innervations. Thirty-six of these dogs received 0.4 mg/kg methoxamine, and three dogs received 1.6 mg/km. The dogs were divided into two groups, one consisted of seventeen dogs with intact vagi, the second being twenty-two vagotomized dogs.

In the dogs with intact vagi methoxamine caused an average decrease in the heart rate of 43.3 ± 3.1 beats/min, ($P < 0.001$). However methoxamine caused a decrease in the heart rate of 20-30 beats/min in only four of the vagotomized dogs.

The decrease in heart rate in the dogs with intact vagi was not related to the incidence of AV-nodal block. Methoxamine caused an average decrease of 31.2 ± 8.6 beats/min in 8 of 17 dogs developing AV-nodal block in response to

p-CMB (20 mg/kg). The nine dogs not developing AV-nodal block had a decrease in the heart rate of 41.1 $\pm$ 7.3 beats/min. The difference between these two groups of dogs was not significant ($P > 0.3$).

(b) The Effect of p-CMB on the Heart Rate

Analysis of the change in heart rate caused by p-CMB (20-80 mg/kg) was carried out in fifty-three dogs, thirty-nine of which were pretreated with methoxamine. A summary of this data is contained in Table VII.

Cumulative doses of p-CMB (20-80 mg/kg) were administered to fourteen dogs not pretreated with methoxamine, six of which had been vagotomized.

Five of the eight dogs with intact cardiac innervation received 20 mg/kg p-CMB. Their heart rates did not change. Three of the six vagotomized dogs received 20 mg/kg p-CMB. Their heart rates decreased by 40 $\pm$ 10 beats/min ($P < 0.05$).

Doses of p-CMB greater than 20 mg/kg decreased the heart rate equally in vagotomized and intact dogs. These fourteen dogs were therefore handled together for the purpose of statistical analysis. Four dogs received p-CMB (30 mg/kg) and showed an average decrease in heart rate of 52.5 $\pm$ 3.6 beats/min ($P < 0.001$); nine dogs received 40 mg/kg and showed an average decrease of 55.5 $\pm$ 8.3 beats/min ($P < 0.001$); and five dogs received 40-80 mg/kg and showed an average decrease

TABLE VII

THE EFFECT OF p-CMB ON THE HEART RATE

Dose of p-CMB mg/kg	NOT TREATED WITH METHOXAMINE				PRETREATED WITH METHOXAMINE			
	Number of dogs	Intact Change in H.R. (range) beats/min	Number of dogs	Vagotomized Change in H.R. beats/min(range)	Number of dogs	Intact Change in H.R. beats/min(range)	Number of dogs	Vagotomized Change in H.R. beats/min(range)
20	5	nil	3	-(30-60)	7	nil	18	nil
					6	≠(20-60)	2	≠(20-60)
					4	-(30-50)	2	-(60)
				Mean-40 ± 10		Mean ≠ 2.9 ± 6.5		Mean -1.9 ± 5.0
				P < 0.05		P > 0.6		P > 0.6
		Intact and Vagotomized Change in H.R. (range) beats/min						
30	1	nil			3	+(20-30)	2	-(30-60)
	3	-(30-120)			2	-(30)		
		Mean -52.5 ± 51				Mean ≠ 4.0 ± 12.3		
		P < 0.05				P > 0.7		
40	9	-(20-90)			1	nil	8	nil
					5	≠(20-50)	6	-(30-60)
					2	-(30)		
		Mean -55.5 ± 8.3				Mean ≠ 12.5 ± 7.5		Mean -17.1 ± 6.1
		P < 0.001				P > 0.1		P < 0.02
> 40	5	-(30-150)			5	-(30-60)	6	-(30-120)
		Mean -66.0 ± 22.4				Mean -42.0 ± 7.4		Mean -60.0 ± 20.9
		P < 0.05				P < 0.01		P < 0.05

of 66.0 $\pm$ 22.4 beats/min ($P < 0.05$).

Cumulative doses of p-CMB (20-80 mg/kg) were administered to seventeen methoxamine-pretreated dogs having intact cardiac innervation. The initial dose was 20 mg/kg in each, neither this nor additional doses up to 40 mg/kg caused consistent changes in the heart rate. Some dogs responded with a bradycardia or a tachycardia whereas others showed no change. Five dogs received doses of p-CMB greater than 40 mg/kg. These dogs demonstrated an average decrease in the heart rate of 42.0 $\pm$ 7.4 beats/min ($P < 0.01$).

Cumulative doses of p-CMB (20-80 mg/kg) were administered to twenty-two methoxamine-pretreated vagotomized dogs. All the dogs received p-CMB (20 mg/kg) as the initial dose. The heart rate was unchanged in eighteen of these dogs. However, in fourteen dogs 40 mg/kg p-CMB caused an average decrease in the heart rate of 17.1 $\pm$ 6.1 beats/min ($P < 0.02$). Doses of p-CMB greater than 40 mg/kg caused an average decrease in the heart rate of 60.0 $\pm$ 20.9 beats/min in five dogs. This also was significant ($P < 0.05$).

The incidence of AV-nodal block after 20 mg/kg p-CMB was not correlated with the above changes in heart rate. Fourteen dogs developing AV-nodal block had an average increase in the heart rate of 4.2 $\pm$ 9.1 beats/min ($P > 0.7$). Twenty-five dogs not developing AV-nodal block had an average increase in the heart rate of 3.2 $\pm$ 3.4 beats/min ($P > 0.4$).

The above results demonstrate that there is no relationship between the changes in heart rate produced by either methoxamine or p-CMB and the incidence of AV-nodal block. This then indicates that effects on the heart rate are not involved in the potentiating effects of methoxamine.

4. Analysis of the Electrocardiographic Changes Produced by p-CMB and Methoxamine

The changes in the P-R interval and the QRS complex on the electrocardiogram were analyzed statistically by Student's t-test, and Student's t-test for paired data. All means are given with their standard errors. The changes caused by methoxamine were taken as the maximum change before the administration of p-CMB. The changes caused by p-CMB were taken as the maximum before AV-nodal block.

(a) The Effect of Methoxamine on the P-R Interval

Analysis of the changes in the P-R interval after methoxamine (0.4 or 1.6 mg/kg) were carried out in a total of thirty-nine dogs which had various cardiac innervations as described in the previous sections.

(i) The Total Group of Thirty-nine Dogs

The P-R interval was lengthened from 10-40 msec in only nine of these dogs; the remaining thirty dogs did not exhibit any change. The nine dogs demonstrating an increase

in P-R interval differed in cardiac innervations. Five dogs were intact, one was vagotomized, two had combined vagotomy and stellate gangionectomy, and one was vagotomized and sham stellate ganglionectomized.

(ii) Intact Dogs

The P-R interval was lengthened in five of the eleven dogs with intact cardiac innervation. The average increase in P-R for all eleven dogs was 10.0 ± 4.0 msec ($P < 0.05$). Four of the five dogs demonstrating this increase in P-R interval received methoxamine 0.4 mg/kg and one 1.6 mg/kg. Four of the six dogs not demonstrating an increase in P-R interval received methoxamine 0.4 mg/kg, the other two receiving the higher dose. Of the five dogs demonstrating an increase in P-R interval, only three developed AV-nodal block to subsequent administration of p-CMB (20 mg/kg). Two of the six dogs whose P-R interval was not lengthened by methoxamine itself developed AV-nodal block to subsequent administration of p-CMB (20 mg/kg).

The above data show that there is no relationship between the increase in P-R interval caused by methoxamine and the incidence of AV-nodal block caused by subsequent administration of p-CMB.

(b) The Effect of p-CMB on the P-R Interval

p-CMB consistently lengthened the P-R interval.

Analysis was carried out to see if methoxamine influenced this increase in P-R interval caused by p-CMB.

The administration of cumulative doses of 20-80 mg/kg p-CMB caused a rapid increase in the P-R interval in fifty-three dogs, thirty-nine of which had been pretreated with methoxamine (0.4 or 1.6 mg/kg). The relationship between the magnitude of this increase in P-R interval and the dose of p-CMB is illustrated in Figure 11. In both groups of dogs the increase in P-R interval in response to 30 mg/kg was no greater than that in response to 20 mg/kg. In the group of dogs not pretreated with methoxamine, 40 mg/kg p-CMB caused an abrupt increase in the P-R interval. However, in the methoxamine-pretreated dogs the increase in P-R interval in response to 40 mg/kg was no greater than that in response to 20 mg/kg. Doses of more than 40 mg/kg caused a large increase in the P-R interval in both groups of dogs. The influence of p-CMB on the P-R interval therefore parallels the AV-nodal blocking effect.

The initial dose of p-CMB (20 mg/kg) was used in analyzing the effect of methoxamine on the increase in P-R interval caused by p-CMB. The increases in P-R interval in response to p-CMB (20 mg/kg) in both the methoxamine-pretreated group of dogs, and the group of dogs not pretreated with methoxamine were significant ($P < 0.001$ and $P < 0.05$, respectively). p-CMB (20 mg/kg) caused a significantly

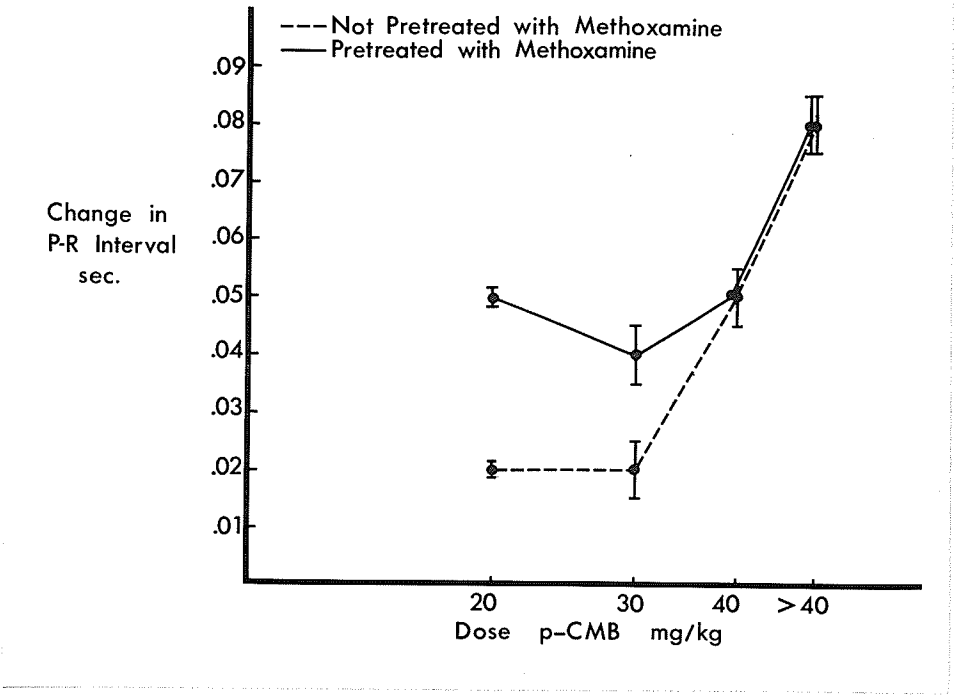

Figure 11. Dose-response curves of the change in P-R interval caused by p-CMB. The average P-R intervals are given with their standard errors. Fourteen dogs not pretreated with methoxamine ------. Thirty-nine dogs pretreated with methoxamine (0.4 or 1.6 mg/kg) ——.

larger increase in the P-R interval in the methoxamine pretreated dogs ($P < 0.05$), despite the fact that methoxamine by itself did not significantly increase the P-R interval.

p-CMB (20 mg/kg) increased the P-R interval without altering the heart rate in twenty-five of the methoxamine-pretreated dogs and five dogs not treated with methoxamine. Therefore the above changes in P-R interval were not due to changes in the heart rate.

The above results indicate that methoxamine potentiates the effects of p-CMB on the P-R interval although it does not have a significant effect itself. The greater incidence of AV-nodal block in the methoxamine pretreated animals was paralleled by a significantly greater increase in P-R interval.

(c) <u>The Effect of Methoxamine and of p-CMB on the Duration of the QRS Complex</u>

The duration of the QRS complex of the electrocardiogram was not significantly altered by either methoxamine or p-CMB.

Methoxamine caused a change in the duration of the QRS complex in only six of thirty-nine dogs. Three of eleven dogs with intact cardiac innervation, and one of six sympathectomized dogs showed an increase, while two of seven sham stellate ganglionectomized and vagotomized dogs showed a decrease.

p-CMB (20-80 mg/kg) caused a change in the duration of the QRS complex in only six of fifty-three dogs. This change was not related to pretreatment with methoxamine, or to the state of the cardiac innervation.

C. DISCUSSION

The results in this section have shown that p-CMB causes AV-nodal block. The incidence of this block was increased by increasing the dose of p-CMB. Vagotomy also appeared to cause an increase in the incidence of AV-nodal block, but this increase was probably attributable, at least in part, to the higher initial doses of p-CMB given to the vagotomized dogs.

The administration of methoxamine before p-CMB increased the incidence of AV-nodal block. This potentiating effect of methoxamine was not due to reflex effects which alter the sympathetic and vagal tone to the heart. Neither stellate ganglionectomy nor vagotomy altered this increased incidence of AV-nodal block. However the combination of vagotomy and stellate ganglionectomy abolished this increase. The combination of vagotomy and sham stellate ganglionectomy also abolished the increased incidence of AV-nodal block. These unusual findings might be explained by the following. The surgical procedure involved in the removal of the stellate ganglia could cause the reflex release of endogenous

catecholamines from the adrenals, heart or other tissues. These catecholamines then could cause an increase in atrio-ventricular conduction, thus antagonizing the depressant effects of p-CMB. However this would not apply to the stellate ganglionectomized group of dogs who had intact vagi, as the vagi could compensate for the effect of the released catecholamines. Therefore a combination of vagotomy and the surgical procedure is necessary to abolish the increased incidence of AV-nodal block caused by methoxamine.

There was no correlation between the pressor response of methoxamine and the incidence of AV-nodal block caused by p-CMB. This indicates that factors other than the pressor response are involved in the potentiating effects of methoxamine.

p-CMB was shown to cause a greater fall in the blood pressure in the methoxamine-pretreated animals. The results tend to indicate that this decrease in blood pressure is due to cardiac depression with subsequent reduction of cardiac output. The action of methoxamine therefore would be to potentiate this depressant effect of p-CMB, resulting in a greater fall in the blood pressure.

The question arises, however, of how much of the fall in blood pressure caused by p-CMB is a result of peripheral vasodilatation. Such vasodilatation could cause venous pooling, thus reducing the cardiac output sufficiently to

decrease the coronary blood flow. If hypoxia of the heart resulted from the reduction in coronary blood flow, vasodilatation could be a contributing factor in the production of AV-nodal block. The fall in blood pressure to a lower level in the methoxamine-pretreated animals may indicate that p-CMB causes a greater decrease in cardiac output and coronary blood flow, and thus produces a more hypoxic heart. It appears possible that the larger fall in blood pressure shown in the methoxamine-pretreated animals could be due to hypovolemia caused by methoxamine. Finnerty (79) showed that noradrenaline caused hypovolemia, while Sutter (80) showed that noradrenaline produced hypovolemia by causing loss of protein-free fluid from the vascular compartment. If p-CMB causes vasodilatation or cardiac depression, then a greater fall in blood pressure would be seen in animals made hypovolemic by methoxamine. However the above studies showed also that a considerable amount of time was involved in order for hypovolemia to develop. p-CMB was administered too soon after methoxamine to allow sufficient time for hypovolemia to develop. Therefore the greater fall in blood pressure in the methoxamine pretreated animals is not likely to be due to hypovolemia.

A number of factors however, are not in accord with hypoxia being the cause of AV-nodal block. A fall in the blood pressure would cause a reflex increase in the sympathetic

tone to the heart, tending to antagonize the depressant effects of p-CMB or hypoxia, rather than enhancing them. Also it has been shown that p-CMB has a direct depressant action on the heart. p-CMB was shown to decrease the heart rate and increase the P-R interval in vagotomized dogs.

Both the fall in blood pressure and the level of blood pressure obtained in response to p-CMB were shown to be related to the incidence of AV-nodal block. However this relationship was influenced by the cardiac innervation of the animals. A high incidence of AV-nodal block was evidenced by both the vagotomized group of dogs and the stellate ganglionectomized group of dogs. However neither of these two groups of dogs showed any relationship between the fall or level of the blood pressure and the incidence of AV-nodal block. Other factors in addition to the blood pressure must therefore be involved in the genesis of AV-nodal block. These results tend to indicate that the fall in blood pressure is a result of a depressed heart, but that the cardiac depression is not always manifested in a fall in blood pressure.

The changes in heart rate produced by either methoxamine or p-CMB were not related to the incidence of AV-nodal block. Therefore the heart rate is probably not one of the factors involved in the potentiating effect of methoxamine.

The electrocardiographic changes produced by p-CMB indicate that p-CMB has a preferential effect on the atrio-

ventricular conduction system. p-CMB was shown to cause a lengthening of the P-R interval without causing any change in the duration of the QRS complex. This indicates that p-CMB causes a depression of atrioventricular conduction without altering intraventricular conduction. However p-CMB produced AV-nodal block without ventricular ectopic foci, indicating that ventricular automaticity is depressed.

Methoxamine did not significantly alter the P-R interval. However a greater increase in the P-R interval in response to p-CMB was obtained in the methoxamine-pretreated animals. These results indicate that methoxamine potentiates the depressant effects of p-CMB while not having any depressant action of its own at that dose. However further investigation (section IV) indicates that methoxamine does depress atrioventricular conduction.

SECTION IV

THE EFFECT OF p-CMB AND METHOXAMINE ON THE FUNCTIONAL REFRACTORY PERIOD OF ATRIOVENTRICULAR TRANSMISSION AND ATRIOVENTRICULAR CONDUCTION TIME

A. INTRODUCTION

A number of reports have indicated that methoxamine has a direct depressant effect on the heart (26,30,33,34,35, 36,37). It therefore seemed possible that the potentiating action of methoxamine reported in the previous section of results was due to a combination of the direct depressant effect of methoxamine and p-CMB on the heart. A change in the refractory period or conduction time in the atrio-ventricular conducting tissue must precede the development of AV-nodal block. Such changes are a graded phenomena, whereas the development of AV-nodal block is all or none. Therefore in order to obtain a more detailed study of the mechanism of methoxamine potentiation, the effects of these two agents were tested on the functional refractory period of atrioventricular transmission (FRP) and atrioventricular conduction time (CT).

The FRP and CT, as well as the effects of various drugs on these parameters have been studied by Krayer et al (72), Kohli (78), Innes et al (81), and Mendez et al (82). The FRP is obtained from the minimal interval between two ventricular impulses which are propagated from the atrium. The FRP is the measurement of the refractory period of that part of the atrioventricular conducting system with the longest refractory period. Therefore the FRP includes the refractory period of the AV-node, the bundle of His with its branches,

and the Purkinje fibers. It also includes the refractory period of the tissue from the atrial muscle to the AV-node, and of the ventricular muscle. CT includes all the steps involved in the spread of an impulse from atrial to ventricular muscle (72).

Mendez (83) showed that the FRP was dependent on the immediately preceding cycle length. Thus the FRP would be longer at slower heart rates. Because of this finding the heart rate was kept constant throughout each individual experiment in this section. This was done by driving the heart at a rate of 20-30 beats/min greater than the initial heart rate.

The general course of an experiment was as follows. One control recording for the measurement of FRP was made during the control period. Ten minutes was allowed between each FRP determination. The various doses of the drug (methoxamine or p-CMB) were injected three minutes prior to the next determination of the FRP. All the data was compared on the basis of the original control run.

All the data in this section were compared by Student's t-test for paired data, and all the means are given with their standard errors.

B. RESULTS

1. The Effect of Increasing Doses of p-CMB on the Functional Refractory Period of Atrioventricular Transmission

p-CMB was administered to ten vagotomized dogs. The initial dose was 5 mg/kg in three dogs; 10 mg/kg in five dogs; and 20 mg/kg in two dogs. Seven of the dogs received additional doses of p-CMB to reach total doses of 20-40 mg/kg.

Cumulative doses of p-CMB (5-40 mg/kg) lengthened the FRP by 4 to 56 msec. The results are shown in Table VIII. The lengthening of the FRP in response to 10, 20, and 30 mg/kg were statistically significant. Six of the seven animals receiving p-CMB (40 mg/kg) developed AV-nodal block at the driving rate and therefore the measurement of the FRP could be made in only one animal.

The average increase in FRP in response to 20 mg/kg was less than the average increase in response to 10 mg/kg. This is probably contributed to by dog #2 which demonstrated a decrease in the FRP in response to 20 mg/kg, and dogs #4 and #6 whose FRP did not increase further when the dose of p-CMB was increased from 10 mg/kg to 20 mg/kg.

Increasing the dose of p-CMB from 10 mg/kg to 30 mg/kg did not cause a significant increase in the prolongation of the FRP. The difference in the mean increases in FRP between

TABLE VIII

THE EFFECT OF CUMULATIVE DOSES OF p-CMB ON THE FUNCTIONAL REFRACTORY PERIOD OF ATRIOVENTRICULAR TRANSMISSION IN VAGOTOMIZED DOGS

Dose of p-CMB mg/kg	Dog #	A Control Functional Refractory Period	B Functional Refractory Period after p-CMB	Difference B-A	
5.0	1	232	240	8.0	
	2	196	200	4.0	
	3	218	212	-6.0	
10.0	4	166	172	6.0	
	5	182	200	18.0	
	6	212	230	18.0	Mean = 17.4
	7	196	215	19.0	SE = 3.2
	8	234	260	26.0	P < 0.01
20.0	9	200	214	14.0	
	10	182	200	18.0	
	2	196	190	-6.0	
	3	218	220	2.0	
	4	166	172	6.0	
	5	182	204	22.0	
	6	212	230	18.0	Mean = 15.7
	7	196	218	22.0	SE = 4.4
	8	234	280	46.0	P < 0.01
30.0	4	166	174	8.0	
	5	182	206	24.0	
	6	212	250	38.0	Mean = 30.8
	7	196	224	28.0	SE = 8.0
	8	234	290	56.0	P < 0.02
40.0	7	196	224	28.0	
	2				
	3				
	4	Measurements were not made as these dogs developed			
	5	AV-nodal block at this dose.			
	6				
	8				

10 mg/kg and 30 mg/kg was not significant ($P > 0.1$).

The FRP in dogs #2 and #3 are misleading because the FRP in response to p-CMB (20 mg/kg) is compared to the control FRP. A larger decrease, and a greater lengthening of the FRP in dogs #2 and #3 respectively will be seen if the FRP in response to 20 mg/kg is compared to the FRP in response to 10 mg/kg instead of the control FRP.

In dog #7 the fall in blood pressure caused by p-CMB was compensated for by occluding the aorta. A dose of 10 mg/kg p-CMB increased the FRP from 196 to 215 msec. The blood pressure was raised mechanically to the control level and the FRP redetermined. It was found to be unchanged at 215 msec. Increasing the dose of p-CMB to 40 mg/kg caused a further increase in FRP to 224 msec, which did not change when the blood pressure was raised back to the control level.

2. <u>The Effect of Methoxamine and of Mechanical Increases in the Blood Pressure on the Functional Refractory Period of Atrioventricular Transmission</u>

Methoxamine (0.4 mg/kg) was administered to thirteen vagotomized dogs. A summary showing the effects of methoxamine and blood pressure on the FRP of these dogs is contained in Table IX. Figure 12 illustrates the effect of methoxamine and mechanical elevation of the blood pressure on the FRP of one of the above dogs.

TABLE IX

THE EFFECT OF METHOXAMINE (0.4 mg/kg AND RAISED BLOOD PRESSURE ON THE FUNCTIONAL REFRACTORY PERIOD OF ATRIOVENTRICULAR TRANSMISSION (FRP) IN VAGOTOMIZED DOGS

FRP IN MILLISECONDS

Dog #	A Control	B After Increasing B.P.	Difference B-A	C After Methoxamine	Difference C-A	D After Methoxamine + Hemorrhage	Difference D-A
11	212	230	18.0	238	26.0		
12	202	220	18.0	256	54.0		
13	262	270	8.0	270	8.0		
14	226			252	26.0		
15	226			266	40.0		
16	224			256	32.0		
17	250			290	40.0	265	15.0
18	210					222	12.0
19	230					256	26.0
20	206					224	18.0
21	220					230	10.0
22	252					260	8.0
23	216					222	6.0
			Mean = 14.6		Mean = 32.3		Mean = 13.5
			SE = 3.3		SE = 5.5		SE = 2.6
			P < 0.05		P < 0.01		P < 0.01

TIME AND HEMORRHAGE CONTROLS

FRP IN MILLISECONDS

Dog #	Control	After Hemorrhage	Difference B-A	After 1.5 hrs.	Difference C-A
24	220	228	8.0	230	10.0
25	216	220	4.0	226	10.0
26	226	230	4.0	230	4.0
			Mean = 5.3		Mean = 8.0
			SE = 1.3		SE = 2.0
			P > 0.05		P > 0.05

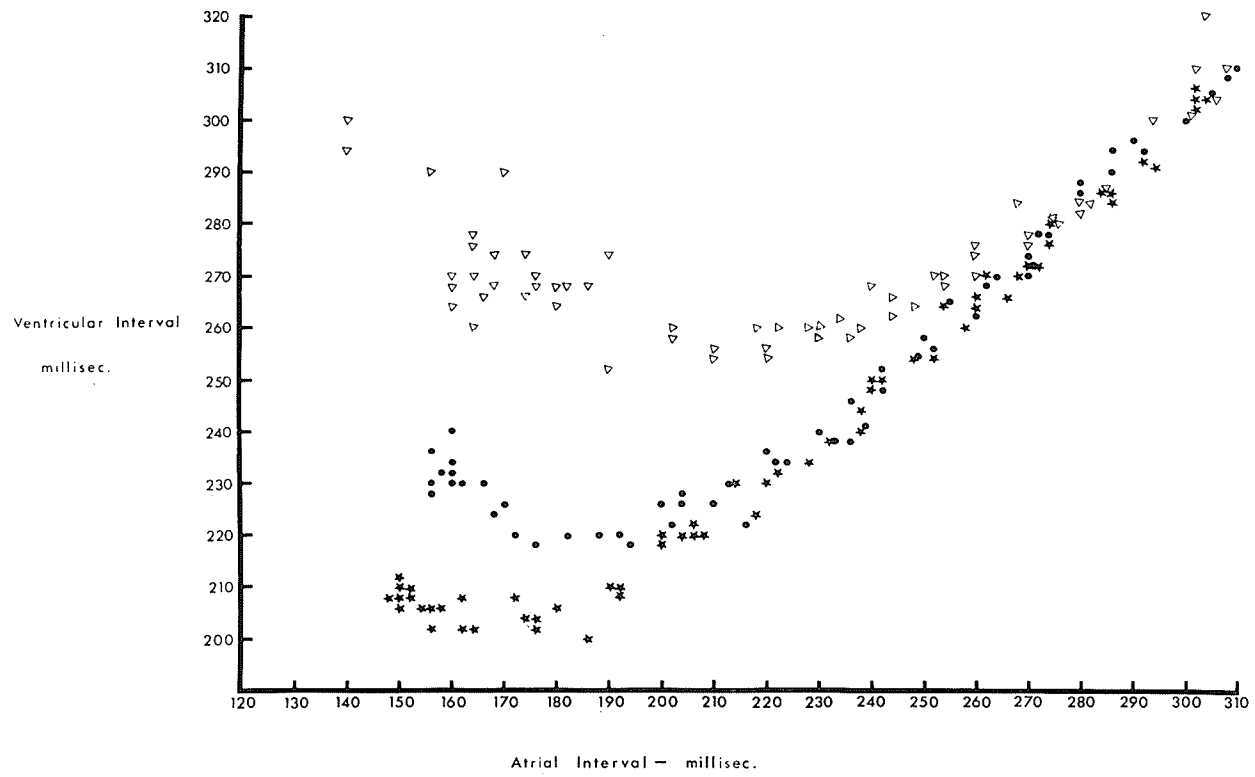

Figure 12. The action of methoxamine and mechanical elevation of the blood pressure on the functional refractory period of atrioventricular transmission. Dog #12-Abscissae: atrial intervals in milliseconds. Ordinates: ventricular intervals in milliseconds. Stars; control observations. Circles: mechanical elevation of the blood pressure. Triangles: methoxamine (0.4 mg/kg).

Methoxamine caused an average lengthening of the FRP of 32.3 ± 5.5 msec in seven dogs, ($P < 0.01$). The increase in blood pressure caused by methoxamine was compensated for in two ways in order to determine if the pressor response to methoxamine was responsible for the above increases in FRP. In three of the thirteen dogs the mean blood pressure (diastolic + 1/3 pulse pressure) was elevated mechanically by 33-60 mm Hg. This was comparable to the pressor response caused by methoxamine. This mechanical increase in the blood pressure caused an average lengthening of the FRP of 14.6 ± 3.3 msec, ($P < 0.05$). In seven of the thirteen dogs the rise in blood pressure caused by methoxamine was prevented by bleeding (50-100 cc). The average lengthening of the FRP in these seven dogs was 13.5 ± 2.6 msec, ($P < 0.01$), but less than the dogs in which the blood pressure rose.

The effects of bleeding alone and of the time taken for these experiments were determined in three dogs. The FRP was not significantly lengthened by bleeding (5.3 ± 1.3 msec, $P > 0.05$), or after 1.5 hours (8.0 ± 2.0 msec, $P > 0.05$).

3. <u>The Effect of p-CMB and of Methoxamine on the Conduction Time of Atrioventricular Transmission</u>

The effects of methoxamine and of p-CMB on the conduction time of atrioventricular transmission were also determined. The delay between the atrial and ventricular responses was measured from the same records used in the determination of the FRP. The data are summarized in

Tables X and XI.

Cumulative doses of p-CMB (5-40 mg/kg) caused an increase in CT from 2 to 52 msec in ten dogs. These increases in CT were statistically significant. The increases in conduction time varied directly with the size of the dose of p-CMB i.e., the larger the dose the greater the increase in CT. The differences in the mean increases in conduction time from 5 mg/kg to 10 mg/kg; 10 mg/kg to 20 mg/kg; and 20 mg/kg to 30 mg/kg; were significant ($P < 0.05$, $P < 0.05$, $P < 0.01$ respectively). Therefore the measurement of CT seems more closely correlated with the dose of p-CMB than is the measurement of FRP. This holds true both for the whole group and for each animal.

In dog #7 the fall in blood pressure caused by p-CMB was compensated for by occluding the aorta. A dose of 10 mg/kg p-CMB increased the CT from 110 msec to 120 msec. When the blood pressure was raised mechanically to the control level and the CT redetermined, it was found to be unchanged at 120 msec. Increasing the dose of p-CMB to 40 mg/kg caused a further increase in conduction time to 148 msec which decreased to 142 msec when the blood pressure was returned to the control level.

Methoxamine (0.4 mg/kg) caused an average increase in CT of 32.5 ± 5.2 msec in seven dogs ($P < 0.001$). However, the CT was not significantly increased in seven dogs when the pressor response to methoxamine was compensated for by bleeding (8.0 ± 3.6 msec, $P > 0.05$). In three dogs, where the blood

TABLE X

THE EFFECT OF CUMULATIVE DOSES OF p-CMB ON THE CONDUCTION TIME OF ATRIOVENTRICULAR TRANSMISSION IN VAGOTOMIZED DOGS

Dose of p-CMB mg/kg	Dog #	A Control C.T.	B C.T. after p-CMB	Difference B-A	
5.0	1	114	118	4.0	Mean = 3.3
	2	122	124	2.0	SE = 0.6
	3	104	108	4.0	P < 0.05
10.0	4	94	110	16.0	
	5	120	126	6.0	
	6	132	154	22.0	Mean = 13.6
	7	110	120	10.0	SE = 2.7
	8	116	130	14.0	P < 0.01
20.0	9	106	132	26.0	
	10	90	102	12.0	
	2	122	132	10.0	
	3	104	130	26.0	
	4	94	128	34.0	
	5	120	160	40.0	
	6	132	160	28.0	Mean = 24.0
	7	110	124	14.0	SE = 3.3
	8	116	142	26.0	P < 0.001
30.0	4	94	134	40.0	
	5	120	160	40.0	
	6	132	184	52.0	Mean = 43.2
	7	110	146	36.0	SE = 3.0
	8	116	164	48.0	P < 0.001
40	7	110	148	38.0	
	2				
	3				
	4	Measurements were not made as these dogs developed			
	5	AV-nodal block.			
	6				
	8				

TABLE XI

THE EFFECT OF METHOXAMINE (0.4 mg/kg) ON THE CONDUCTION TIME OF ATRIOVENTRICULAR TRANSMISSION IN VAGOTOMIZED DOGS

CONDUCTION TIME IN MILLISECONDS

	A	B		C		D	
Dog #	Control	After Increasing B.P.	Difference B-A	After Methoxamine	Difference C-A	After Methoxamine ≠ hemorrhage	Difference
11	108	140	32	150	42		
12	108	120	12	158	50		
13	124	158	34	170	46		
14	120			142	22		
15	140			154	14		
16	104			132	28		
17	100			126	26	130	30
18	136					142	6
19	108					110	2
20	106					108	2
21	106					110	4
22	156					160	4
23	100					108	8
			Mean = 26.0		Mean = 32.5		Mean = 8.0
			SE = 7.1		SE = 5.2		SE = 3.6
			P > 0.05		P < 0.001		P > 0.05

TIME AND HEMORRHAGE CONTROLS

CONDUCTION TIME IN MILLISECONDS

	A	B		C	
Dog #	Control	After Hemorrhage	Difference B-A	After 1.5 Hours	Difference C-A
24	112	114	2	114	2
25	68	90	22	78	10
26	100	80	-20	82	-18
			Mean = 1.3		Mean = 2.0
			SE = 11.6		SE = 2.3
			P > 0.9		P > 0.4

pressure was elevated mechanically, the CT also was not significantly increased (26.0 $\pm$ 7.1 msec, $P > 0.05$). However, this lack of significance is probably due to the small number of experiments.

The effects of bleeding alone and of the time taken for these experiments were determined in three dogs. The CT was not significantly increased by bleeding (1.3 $\pm$ 11.6 msec, $P > 0.9$), or after 1.5 hours (2.0 $\pm$ 2.3 msec, $P > 0.4$).

C. DISCUSSION

This section of the results have shown the p-CMB causes a prolongation of the FRP and an increase in CT. The results further indicate that these changes are due to a direct depressant action of p-CMB on the atrioventricular conduction system.

Section III showed that p-CMB caused a fall in the blood pressure. As previously discussed if this fall in blood pressure resulted in anoxia, it could contribute to the prolongation of the FRP and increase in CT. However, a number of factors indicate that this fall in blood pressure is not a contributing factor in the prolongation of the FRP or increase in CT. A fall in the blood pressure would cause a reflex increase in sympathetic tone to the heart and thus decrease the FRP and CT. In the three control animals, where the blood pressure was decreased by bleeding, neither the CT nor the

FRP were significantly altered. In one experiment the prolongation of the FRP and increase in CT caused by p-CMB were not altered when the blood pressure was returned to the control level. These factors, therefore, indicate that the prolongation of the FRP and the increase in CT caused by p-CMB are the result of a direct effect of the drug on the atrioventricular conduction system of the heart.

Both the direct effect of methoxamine and its pressor response caused comparable lengthening of the FRP. In three experiments the mechanical elevation of the systemic blood pressure was shown to cause a prolongation of the FRP. However, methoxamine was shown to cause a larger increase in the FRP than a comparable increase in the blood pressure in two of three experiments. The increase in the FRP response to methoxamine was reduced when the pressor response was prevented by bleeding.

The prolongation of the FRP caused by methoxamine therefore, involves both a direct effect of methoxamine on the heart and an increase in blood pressure. However, the increased blood pressure could lengthen the FRP by a direct effect on the heart, or decrease the reflex sympathetic tone to the heart. Kohli (78) has shown that mechanical elevation of the blood pressure in vagotomized spinal dogs causes a prolongation of the FRP. In addition, Innes (84) showed that mechanical elevation of the blood pressure in the dog heart-

lung preparation caused a prolongation of the FRP. These findings tend to indicate that the increased blood pressure prolongs the FRP by a direct effect on the heart, rather than by decreasing the sympathetic tone.

Methoxamine caused an increase in the total CT from the atria to the ventricles. However the CT was not significantly increased when the blood pressure was elevated mechanically. This lack of statistical significance is probably due to the small number of experiments. When the pressor response to methoxamine was prevented by bleeding, the increase in CT was reduced greatly and was no longer statistically significant. These results indicate that the direct effect of methoxamine and the pressor response to this drug are necessary to cause a significant increase in the conduction time of atrioventricular transmission.

Gilbert (30) showed that methoxamine caused a large increase in the atrioventricular conduction time and a small increase in the "absolute refractory period" of vagotomized open chest dogs. He attributed these changes to a decreased excitability of the cardiac tissue. However he did not take the pressor response to methoxamine into consideration. In view of the above findings it is possible that some of the increase in conduction time and "absolute refractory period" were due to the pressor response.

SECTION V

GENERAL DISCUSSION

GENERAL DISCUSSION

The results indicate that p-CMB causes atrio-ventricular nodal block by a direct depressant effect on the nodal tissue. The AV-nodal block produced by p-CMB is not due to increased vagal activity, because vagotomy did not decrease the incidence of block. A number of conflicting results have been obtained as to the role of the vagus in the cardiac toxicity produced by organic mercurials. Jackson (4) suggested that the organic mercurials produce cardiac toxicity by increasing the activity of the vagus. However other authors (1,3,6) have indicated that neither the parasympathetic nor sympathetic innervation of the heart contribute to the cardiac toxicity of the organic mercurials.

The results further indicate that p-CMB depresses both atrioventricular conduction and ventricular automaticity. p-CMB caused a lengthening of the P-R interval without altering the duration of the QRS complex. These findings are in agreement with those of Farah *et al* (9) who suggested that p-CMB interferes mainly with atrioventricular conduction without altering intraventricular conduction. p-CMB also depressed ventricular automaticity, since the appearance of ventricular ectopic beats was rare. This finding is in contrast to those obtained with other organic mercurials which cause ventricular ectopic beats, ventricular tachycardia, and ventricular

fibrillation (5,6,8,9).

Further evidence for the direct effect of p-CMB on the atrioventricular conducting system was obtained from the studies on the functional refractory period and conduction time. p-CMB prolonged the FRP and increased atrioventricular CT.

Near maximal prolongation of the FRP was reached with small doses of p-CMB. Very large doses prolonged the FRP to the extent that AV-nodal block was produced at the fast driving rates used in these experiments. Alanis et al (63) and Hoffman et al (73) have suggested that the delay in atrioventricular transmission can take place at several sites in the atrioventricular conducting system. In addition, Krayer et al (72) pointed out that the FRP is the measurement of the refractory period of that part of the atrioventricular conducting system with the longest refractory period. Therefore a small dose of p-CMB could affect the tissue with the longest refractory period, while a larger dose could affect other tissue with a shorter refractory period. The effect of the larger dose therefore would not be seen. p-CMB then would not be expected to affect the FRP of the atrioventricular conducting tissue in a systematic way, or affect all the tissues simultaneously. It is therefore not surprising that increasing doses of p-CMB do not have an additive effect in prolonging the FRP. At the driving rates used in

these experiments the effect of p-CMB on the FRP approaches a maximum at a time when further increases in dose do not yet cause AV-nodal block. Because of this finding the ability of p-CMB to cause AV-nodal block cannot be predicted from its effect on the FRP.

The measurement of conduction time, however, showed a linear dose-response relationship, with larger doses of p-CMB causing a greater increase in conduction time. The conduction time of atrioventricular transmission is the measurement of the time involved in the conduction of an impulse through the entire atrioventricular conducting tissue and not just one segment as in the measurement of FRP. Therefore a linear dose-response relationship would be expected.

The results show that methoxamine potentiates the depressant effects of p-CMB. Methoxamine increased the incidence of AV-nodal block caused by p-CMB. The prolongation of the P-R interval caused by p-CMB was also potentiated by methoxamine. In addition the fall in blood pressure caused by p-CMB was potentiated by methoxamine. These findings are not surprising in view of a number of reports (26,33,34,35,36,37) that methoxamine causes cardiac depression.

There are two possible mechanisms by which methoxamine could be exerting its potentiating effect. (1) The rise in blood pressure caused by methoxamine could exert an inhibitory effect on the atrioventricular conducting system. This could

occur by reflex changes in vagal and sympathetic tone to the heart. In addition the increased blood pressure could have a direct inhibitory effect on the atrioventricular conducting tissue. (2) Methoxamine could be exerting a direct inhibitory effect on the atrioventricular conducting system of the heart.

The present results indicate that the pressor response to methoxamine may be involved in the potentiation of the depressant effects of p-CMB. However the reflex changes in sympathetic and vagal tone to the heart caused by the pressor response to methoxamine are not involved in this potentiation. This is evidenced by the fact that the removal of either sympathetic or vagal tone to the heart did not alter the increased incidence of AV-nodal block caused by methoxamine.

However, as discussed previously, either mechanical elevation of the blood pressure or the pressor response to methoxamine prolonged the FRP. The increase in blood pressure caused by methoxamine was also shown to be involved in increasing the conduction time of atrioventricular transmission. There are two possible mechanisms by which these changes could be brought about. Firstly, the increase in blood pressure could cause distention and stretching of the atrioventricular conducting tissue and thus impair impulse propagation. Secondly, the increased blood pressure could cause a decrease in the sympathetic tone to the heart. However as previously

discussed in Section IV the pressor response to methoxamine prolongs the FRP by a direct effect on the heart rather than by decreasing the sympathetic tone. These increases in FRP and CT indicate that the conducting tissue is depressed. Under this condition the atrioventricular conducting system might be more susceptible to the effects of p-CMB and result in a greater fall in blood pressure and a greater prolongation of the P-R interval. However the increased incidence of AV-nodal block would not be influenced by the pressor response to methoxamine as the blood pressure is down to low levels at the time that p-CMB causes AV-nodal block. The lack of correlation between the pressor effects of methoxamine and the incidence of AV-nodal block also suggests that factors other than the pressor response are involved in the potentiating effects of methoxamine.

Evidence has also been presented to show that methoxamine has a direct inhibitory effect on the heart independent of its pressor action. This inhibitory effect could contribute to the potentiation of the depressant effects of p-CMB. It was suggested previously in Section III that the fall in blood pressure caused by p-CMB is a result of cardiac depression. Methoxamine was shown to potentiate this fall in blood pressure, and therefore could also be causing cardiac depression.

Further evidence in support of the direct depressant

effect of methoxamine was obtained from the electrocardiographic studies. Methoxamine potentiates the lengthening of the P-R interval caused by p-CMB. It is therefore possible that the potentiating effect of methoxamine is a result of direct depression of the atrioventricular conducting system.

The results obtained from the FRP and CT studies also indicate that methoxamine has a direct inhibitory effect on the atrioventricular conducting system. Both the FRP and CT were increased by methoxamine independent of its pressor response. Increases in these parameters are an indication of cardiac depression. Therefore the direct depressant effect of methoxamine could contribute to the depressant effects of p-CMB, and thus increase the incidence of AV-nodal block.

Imai et al (50) suggested that methoxamine is a beta adrenergic blocking agent similar to dichloroisoproterenol. They postulated that the cardiac depressant effects of methoxamine were a result of a competitive antagonism between methoxamine and intrinsic catecholamines stored in the myocardium and released in small quantities to maintain the normal heart rate and contractility. This hypothesis is consistent with the findings presented here, and therefore the direct inhibitory effect of methoxamine could be due to such an antagonism.

The foregoing evidence shows that p-CMB causes AV-nodal block by depressing atrioventricular conduction. This

depressant effect of p-CMB is potentiated by methoxamine in two ways. Firstly by the direct cardiac inhibitory effect of methoxamine, and secondly by the direct cardiac inhibitory effect of the pressor response to methoxamine.

SECTION VI

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

p-CMB (20-80 mg/kg) caused AV-nodal block in pentobarbital-anaesthetized dogs. Methoxamine (0.4 or 1.6 mg/kg) given before p-CMB increased the incidence of AV-nodal block. This potentiating effect of methoxamine was not due to reflex effects which alter sympathetic and vagal tone to the heart.

Changes in the blood pressure, heart rate, and electrocardiographic recordings due to methoxamine and p-CMB were analyzed to see if they were related to the blockade of atrioventricular conduction.

The pressor response to methoxamine was not correlated with the incidence of AV-nodal block. This indicated that factors other than the pressor response are involved in the potentiating effect of methoxamine.

p-CMB (20-80 mg/kg) lowered the systemic arterial blood pressure. p-CMB (20 mg/kg) caused a significantly greater fall in the methoxamine-pretreated dogs. The fall in blood pressure was significantly greater and the final level of the blood pressure was significantly lower in those dogs subsequently developing AV-nodal block after p-CMB (20 mg/kg). This was not the case in dogs without vagal or sympathetic innervation to the heart.

There was no relationship between changes in heart

rate from either methoxamine or p-CMB and the incidence of AV-nodal block. This suggested that the heart rate is not involved in the potentiating effects of methoxamine.

p-CMB (20-80 mg/kg) significantly lengthened the P-R interval without altering the duration of the QRS complex on the electrocardiogram. Methoxamine (0.4 or 1.6 mg/kg) did not significantly alter either the P-R interval or the QRS complex. However a significantly greater increase in the P-R interval in response to p-CMB (20 mg/kg) occurred in dogs given methoxamine (0.4 or 1.6 mg/kg) beforehand.

A more detailed study on the mechanism of methoxamine potentiation was carried out by testing the effects of p-CMB and of methoxamine on the functional refractory period of atrioventricular transmission (FRP) and atrioventricular conduction time (CT). Methoxamine (0.4 mg/kg) or p-CMB (5-40 mg/kg) prolonged the FRP and increased the CT. When the increase in blood pressure due to methoxamine was prevented the prolongation of the FRP and increase in CT was less. Mechanically induced increases in blood pressure significantly prolonged the FRP but did not increase the CT.

It is concluded that p-CMB causes AV-nodal block by depressing atrioventricular transmission. This depressant effect of p-CMB was potentiated by methoxamine in two ways. First, by the direct depressant effect of methoxamine and secondly, by the rise in blood pressure caused by methoxamine.

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