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.

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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