Cardiac Actions of Methoxamine

With Special Reference to Its Antagonistic Action to Epinephrine

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Since the introduction of methoxamine as a sympathomimetic vasoconstrictor drug, its peculiar cardiac actions have attracted some attention. According to Stutzman et al., it produced neither tachycardia nor arrhythmia in the dog heart sensitized with cyclopropane. Lahti et al. reported similar effects during chloroform anesthesia and in animals with experimentally induced myocardial infarcts. Melville and Lat described the depressant action of this compound on the isolated perfused rabbit heart. Recently, Hashimoto et al. reported that methoxamine reduced the oxygen consumption and the coronary blood flow in the isolated fibrillated dog heart.

The present study pertains to the basic character of the depressant action of methoxamine on the dog heart-lung preparation. Special attention was directed toward the antagonistic action of this compound to epinephrine.

Methods

Thirty-three heart-lung preparations (HLP) were carried out according to the Krayer-Mendez modification of the original Starling method. Male dogs weighing around 10 Kg. were used. They were anesthetized with sodium pentobarbital (35 mg./Kg.) administered intraperitoneally. A mixture of 5 per cent carbon dioxide in oxygen was used for artificial ventilation of the lung. The defibrinated blood needed for the HLP was obtained from other large dogs under thiopental anesthesia. The total blood volume present in the HLP at the beginning varied between 1,200 and 1,600 ml. Its level in the venous reservoir was kept constant at 100 mm. above the opening of the inferior vena cava. While temperature of the blood varied from experiment to experiment over the range of 37 to 38.8 C., great care was taken to maintain uniform temperature throughout each individual experiment; temperature changes were not greater than ± 0.3 C. in the course of any experiment. To maintain the blood sugar at normal level, about 500 mg. of glucose were added to the circulating blood at the time the preparation was set up, and then every 10 or 15 minutes 80 to 100 mg. of glucose were added.

By adjusting the arterial resistance, the mean arterial pressure (recorded by a mercury manometer) was set at approximately 100 mm. Hg.

The systemic cardiac output (total output of left ventricle minus coronary flow) was measured by a Weese stromuhr. It was initially set at a value between 350 and 500 ml. per minute. The ability of the heart to handle an increase in blood supply was selected as the principal criterion for estimating cardiac competence. It was determined according to the procedure of Krayer by raising the level of the blood in the venous reservoir by 50 mm. and measuring the rise of the right atrial pressure. The results of these determinations have been expressed as the competence index of the heart, which is the ratio:

\[
\frac{50 - \text{increase in right atrial pressure (mm. H}_2\text{O)}}{50}
\]

The heart rate was counted from electrocardiographic records obtained with an ink-writing oscillograph. Coronary-sinus outflow was channeled into a Morawitz cannula, measured by an automatically recording bubble flowmeter, and returned to the circulation via the inferior vena cava. The value thus obtained was taken to be 60 per cent of the total coronary flow. The pulmonary arterial pressure was recorded from one of the arterial branches of the cardiac lobe of the lung, using a bromoform manometer.

Synthetic i-epinephrine (Sankyo) was administered in the form of hydrochloride. In several experiments, cardioacceleration was produced by continuous infusion by means of an automatically driven syringe delivering about 4 \( \mu \)g. of epinephrine per minute. Methoxamine hydrochloride (Vasoxyl, Burroughs Wellcome) was given by single injections over a period of about two minutes to achieve uniform distribution. Veratrac-
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mine was brought into solution with 0.2 ml. of tenth-normal hydrochloric acid for every 5 mg. of the alkaloid and made up to a concentration of 1:1,000 with distilled water. To exclude vagal effects originating in the peripheral parasympathetic neuron in the heart, 10 mg. of atropine were given in every experiment. All doses refer to the bases, except in the case of atropine, the dose of which refers to the sulfate.

Results

Cardiodepressant Action of Methoxamine

Figure 1 represents a typical experiment showing the effects of methoxamine on the HLP. After control measurements were made, increasing doses of methoxamine were administered as illustrated in figure 1. In doses below 1 mg., there were no significant changes observed either in the right atrial pressure or in the systemic output. When cumulative doses amounted to 4 mg. or more, a decrease in the systemic output and a rise of the right atrial pressure with a visually observable dilatation of the ventricles occurred. Throughout this course, the heart retained its regular sinus rhythm and the heart rate decreased relatively little.

Three minutes after each administration of methoxamine, the level of the blood in the venous reservoir was elevated by 50 mm. to test the myocardial competence. The resultant rise of the right atrial pressure gives a measure of the cardiac contractility (fig. 1). Simultaneously with these changes, a moderate decrease in the coronary flow and a rise of the pulmonary arterial pressure were observed (fig. 1).

In table 1 are summarized the numerical data on the effects of 20 to 30 mg. of methoxamine on the various aspects of the cardiac activity.

Antagonistic Action of Methoxamine to Epinephrine

Modification of the Chronotropic Effect of Single Doses of Epinephrine

When 2 to 5 μg. of epinephrine were injected in the ILLP of the dog, the heart rate increased considerably from the initial level of the denervated heart to reach a peak in 20 to 30 seconds, and then gradually decreased to the initial level. A second injection of epinephrine proved to be as effective as the first in its cardioaccelerator action; the same degree of increase in the heart rate occurred, and the time course was practically unchanged. However, after administration of 10 to 20 mg. of methoxamine, the heart became less sensitive to the cardioaccelerator action of epinephrine. Figure 2 shows that administration of 2 μg. of epinephrine increased the heart rate from 127 to 175 (increase of 38 per cent). Nine minutes after 20 mg. of methoxamine, the same dose increased the heart rate from 128 to 143 (increase of 12 per cent). An additional 10 mg. of methoxamine further weakened the responsiveness: 2 μg. of epinephrine increased the heart rate from 127 to 138 (increase of only 9 per cent).

Effect of Methoxamine on the Cardioaccelerator Action of Continuous Epinephrine Infusion

When epinephrine was administered continuously at a rate of 2 to 4 μg. per minute...
in the HLP of the dog, the heart rate increased gradually to a steady level, its height depending on the rate of administration. As a rule it took about 30 minutes to reach that level.

As is illustrated in figure 3, administration of increasing doses of methoxamine brought about a stepwise decrease in the heart rate, which had been augmented by continuous infusion of epinephrine. Finally, when the cumulative dose amounted to 20 mg., the heart rate was reduced to close to the initial value. The steepest fall in the heart rate took place during the first two or three minutes, but some further deceleration usually occurred during the subsequent 5 to 10 minutes until a new level was reached.

Although markedly reduced by methoxamine, the responsiveness of the heart to the accelerator action of epinephrine was not entirely abolished by this compound. While a certain infusion rate of epinephrine may no longer increase the heart rate, a sufficiently high concentration of epinephrine reaching the heart still qualitatively retains its characteristically positive chronotropic effect. Thus, 10 times as much as the initial infusion rate of epinephrine readily overcame the reduced sensitivity of the heart and led to a very marked increase in the heart rate (fig. 3).

Inotropic Action

Doses of methoxamine which markedly reduced the positive chronotropic effect of epinephrine interfered just as well with the inotropic effect of this compound. When 2 µg. of epinephrine were given at about 10 minutes after 30 mg. of methoxamine, both the increase in the systemic output and the fall of the right atrial pressure were smaller than those caused by the same dose of epinephrine in the control period, although the myocardial failure had been induced by methoxamine (figs. 4a and 5). If methoxamine does not interfere with the positive inotropic action of epinephrine, the same dose of epinephrine given to a failed heart with a high right atrial pressure and a decreased systemic output should bring about a greater response associated with a greater increase in the systemic output and a drastic fall of the right atrial pressure, which is the usual behavior of this sort of preparation. An example of this phenomenon is presented in figures 4(b) and 5, in which the failure was induced by 80 mg. of sodium pentobarbital.

Since the output per minute is the product of the stroke volume and the heart rate per minute, it seems probable that the increase in the output may, in certain circumstances, be largely due to the increase in the heart rate. If this is true of the effects of epinephrine in HLP, the reduced inotropic response to epinephrine of the methoxamine-treated heart may be due, at least in part, to the inhibition of the characteristic chronotropic effect by methoxamine. Using veratramine, we examined the point. In figures 4(c) and 5 are shown records of such an experiment, in which 0.5 mg. of veratramine was administered to the heart with the spontaneously
developed failure after the lapse of three hours. While veratramine in itself had no remarkable effect except a moderate decrease in the heart rate, the responsiveness of the heart to the cardioaccelerator action of epinephrine was greatly reduced by this agent. Nevertheless, the positive inotropic action of epinephrine remained unaffected, and as the heart had deteriorated, the inotropic response elicited was far greater than it was in the control period. Such being the case, it must be admitted that the positive inotropic effect of epinephrine is due entirely to the increase in the stroke volume, and we must conclude that methoxamine has an inhibitory action on the inotropic effect of epinephrine, besides its inhibitory action on the chronotropic effect.

The antagonistic effect of methoxamine on the positive inotropic action of epinephrine was also observed during the experiments with continuous infusion of epinephrine. As is demonstrated in figure 6, the increase in the systemic output and the reduction of the right atrial pressure produced by continuous infusion of epinephrine (4 μg./min.) were surmounted stepwise by graded doses of methoxamine, although the infusion was going on. It seems that the same quantitative relationship observed with the chronotropic action prevails also in this phenomenon: 10 times the initial infusion rate of epinephrine easily overcame the effect of methoxamine, and the systemic output again increased and right atrial pressure dropped definitively.

Discussion

The observations reported in this paper indicate that methoxamine can annul both the positive inotropic and the positive chronotropic actions of epinephrine. Since all the experiments were carried out in the isolated atropinized heart, it may be natural that we should hold the site of action to be in the heart independent of its parasympathomimetic innervation.

In view of the similarity in the chemical structures of epinephrine and methoxamine, we must first of all take into account the possibility of the specific antagonism at the receptor site. It is possible that methoxamine combines so firmly with the receptor responsible for the positive inotropic and the positive chronotropic actions of epinephrine that epinephrine is repelled and its action almost prevented. According to Ahlquist, the receptor in question must be classified as β, inasmuch as isoproterenol is a potent stimulant. If we accept his classification, according to the above-postulated mechanism of action of methoxamine, it may well be that we are confronted with another example of a blocking agent of adrenergic β receptor, such as ephedrine and DCI (dichloro analogue of isoproterenol; 1-[3', 4'-dichlorophenyl]-2-isopropyl-aminoethanol). This blocking action, as it exists, might be a competitive antagonism, for 10 times the initial dose of epinephrine can produce a marked positive inotropic and chronotropic action even in the presence of methoxamine.

At present, two known facts favor the idea of competitive antagonism at the place of the β receptor as stated above: the one relates to the action of methoxamine on the tracheal smooth muscle and the other to the action of that compound on the vascular smooth muscle.

A blockade of the adrenergic β receptor of the tracheal smooth muscle by methoxamine was observed in our laboratory (data to be published in the near future). While methoxamine in a dose of 100 μg./ml. produced practically no change in the tonus of the guinea pig's tracheal smooth muscle, it selectively blocked the relaxation of the muscle by 1 μg./ml. of epinephrine. This is the outcome of a specific blocking action of methoxamine on the adrenergic β receptor, for a successive administration of 100 μg./ml. of theophylline ethylenediamine still caused a typical relaxation.

The recent report of Levy and Ahlquist might be taken as additional corroboration, although they themselves did not interpret their findings in that light. They have demonstrated that the depressor effect of epinephrine elicited by 10 mg./Kg. of dibenamine
The effect of methoxamine on the positive chronotropic action of single doses of epinephrine. Dog, male, 6 Kg. (exp. 28). Mean arterial pressure 104 mm. Hg, systemic output 480 ml./min. Total blood volume at the beginning, 1,500 ml. Temperature was 37.2 ± 0.3 C. Control: the response of the HLP to 2 fxg. of epinephrine prior to giving methoxamine. Other two curves: the response to the same dose of epinephrine after 20 to 30 mg., respectively, of methoxamine (Meth).

The inhibitory effect of graded doses of methoxamine on the cardioacceleration caused by continuous infusion of epinephrine. Dog, male, 9 Kg. (exp. 11). Mean arterial pressure 99 mm. Hg, systemic output 450 ml./min. Total blood volume at the beginning was about 1,440 ml. Temperature was 38.1 ± 0.3 C. Methoxamine was administered in increasing doses as indicated. The doses in parentheses represent the total amounts of methoxamine administered up to the time of the respective signals. Atropine (Atr), epinephrine (Aep), methoxamine (Meth), temperature (Temp).

In the absence of extrinsic epinephrine, a marked, negative inotropic action with a slight decrease in the heart rate was observed with methoxamine. Since methoxamine has two sites of action outside the heart even in the HLP, e.g., the coronary artery and the pulmonary artery, which may be responsible for the observed impairment of the cardiac contractile force, we will consider first these extracardiac factors.

It is generally admitted that when moderate cardiac outputs are maintained at mean aortic blood pressures close to 100 mm. Hg, the oxygen requirements of the HLP of the dog are of the order of magnitude of 200 to 500 ml. per 100 Gm. of heart per hour. As a matter of course, the requirements are met by the arterial blood flowing through the coronary circuit. An oxygen content of the arterial blood of the HLP ranging from 15 to 22 per cent, a rate of coronary flow of about 60 ml. per 100 Gm. of heart per minute, seems to be a requisite for the normal functioning of this sort of preparation, provided that at the limiting conditions practically all the oxygen is taken in from the blood and utilized. In the present series of experiments, the rate of coronary flow was, for the most part, so ample (average 129 ml./100 Gm. of heart/min.) that the reduction of the coronary flow had to run up to as much as 60 per cent before the actual value of the flow reached the critical value. In terms of this observation, the decrease in the coronary flow produced...
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by methoxamine was trivial, the decreased value remaining far above the critical value, and we cannot but consider it a factor of secondary importance in precipitating the observed failure in the contractile force.

The increase in the pulmonary arterial pressure induced by methoxamine may be due either to the increase in the resistance of the pulmonary vessels or to the rise in left atrial pressure. Since the latter was not measured and since methoxamine has been shown to be a poor vasoconstrictor for the lung vessels, the most probable cause of rise in pulmonary arterial pressure is a rise in left atrial pressure.

Moreover, according to the recent report of Guyton et al., the ability of the right ventricle to compensate is relatively good, a rise of pulmonary arterial pressure on the order of 300 per cent being necessary to produce a perceptible rise in the right atrial pressure. The rise in the pulmonary arterial pressure produced by methoxamine in most of our cases was less than 40 per cent, a value far below the magnitude of 300 per cent indicated above.

In any event, it may be improper to denote the rise in the pulmonary arterial pressure as the main cause of the observed impairment of the cardiac contractile force.

Thus, excluding the two extracardiac factors, we now return to the direct action on the myocardium.

In recent years, increasing evidence has been accumulated indicating that certain amounts of norepinephrine or epinephrine are stored in the myocardium and released incessantly in small quantities to maintain the normal heart rate and contractility, thereby serving as "humoral" agents for the regulation of cardiac function. Since methoxamine has an antagonistic action to epinephrine, the negative inotropic, as well as the negative chronotropic actions of methoxamine might be explainable, at least in part, in terms of antagonism to such sympathomimetic amines. In this respect, it would be interesting to examine the effect of methoxamine on the heart depleted of intrinsic symp-
The effects of epinephrine on the cardiac output and the heart rate. Sodium pentobarbital (PB), veratramine (V), methoxamine (Meth), heart rate (HR). (a) In the pentobarbital-induced failure. (b) In the spontaneously failing heart to which veratramine was administered. (c) In the heart treated with methoxamine. The same experiments as in figure 4. (Shaded bars)—the systemic output before epinephrine; (open bars)—the systemic output after 2 µg. of epinephrine; (closed circles connected with a broken line)—the heart rate per minute.

amounts of norepinephrine and epinephrine after the animal has been treated with appropriate doses of reserpine.)

Several such experiments were performed in our laboratory using the HLP made from the dogs to which 0.1 mg./Kg. of reserpine had been administered intraperitoneally 24 hours before the experiments. The results were as follows: The slight decrease in the heart rate induced in the normal HLP by 4 to 10 mg. of methoxamine was not found in the HLP of the treated animals, while the impairment of contractile force was observed practically unchanged or even potentiated. Such being the case, it may be proper to conclude that the heart-rate decrease induced by 4 to 10 mg. of methoxamine is due principally to the antagonistic action of methoxamine to the "humoral" effects of the intrinsic sympathomimetic amines. As for the impairment of the contractile force, we must take into consideration at least an additional mechanism other than the antagonistic action against the intrinsic sympathomimetic amines.

When we come to think about the precise nature of this mechanism, there are at present two findings to serve as the starting point for discussion.

To begin with, we must turn our attention to the nonspecific stabilizing action of methoxamine. Studying the effects of this compound on the transmembrane potential of the guinea pig's ventricular muscle, with the aid of the glass capillary ultramicroelectrode, one of the authors has found that methoxamine in a dose of 100 µg./ml. reduced the maximum rate of rise of the rising phase of the action potential remarkably, without producing any concomitant change in the magnitude of the membrane resting potential. The rising phase of the action potential is the reflection of the considerable rise in Na permeability, and this action of methoxamine may be interpreted as a selective depression of the rise in Na permeability at this phase. A similar effect was reported with cocaine, procaine, antihista-
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amines, and quinidine, and the term "stabilizer" was given to these agents.

In the second place, the following fact must be taken into consideration. The recent report from our laboratory has shown that methoxamine reduced the oxygen consumption of the isolated perfused dog heart with experimentally induced fibrillation. These data could be an expression of the inhibition of tissue respiration. Dealing with the protective action of methoxamine against the toxic effects of x-ray irradiation, Smith et al., in their discussion, have suggested the reduction of metabolic rate as the possible mechanism of this protection. If methoxamine has an inhibitory action on the tissue metabolism, as these data imply, it cannot be ignored in an adequate evaluation of the negative inotropic action of methoxamine.

These two findings, i.e., the stabilizing action and the inhibitory action on tissue metabolism, might have nothing to do with each other, and each might in its own way be the cause of an impairment of the heart contractile force. But another possibility is not easily overlooked: In further analyses the one might be unmasked and looked upon as a mere outcome of the other. For example, the stabilizing action might be nothing but an expression of the inhibition of tissue metabolism, and the latter might be the most fundamental cause of the negative inotropic action of methoxamine or vice versa. Shanes, in his extensive review (1958), has called particular attention to the fact that the metabolic inhibition often brings about a change in the membrane potential which is almost indistinguishable from that of stabilization. We have attempted to elucidate this point, but for the present we are not in a position to settle it once and for all.

Summary

The cardiac action of large doses of methoxamine and its antagonistic action to epinephrine were studied, using a dog heart-lung preparation. In doses of 4 mg. and above, methoxamine showed a marked negative inotropic action, while it produced only a slight decrease in the heart rate. Pretreatment of animals with 0.1 mg./Kg. of reserpine did not modify the inotropic action, but the decrease in the heart rate disappeared. Simultaneous with these changes, a decrease in the coronary flow and a rise of the pulmonary arterial pressure were observed. Methoxamine in doses of 1 mg. and more abolished both the positive inotropic and the positive chronotropic actions of epinephrine. The negative inotropic action of methoxamine was ascribed to a nonspecific, as yet undetermined mechanism, and the antagonistic action was ascribed to the competitive antagonism at the receptor site (thus methoxamine may be looked upon as a blocking agent of adrenergic β receptor). The weak negative chronotropic action was taken to be an expression of the blockade of the humoral effects of the intrinsic sympathomimetic amines.

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