Inotropic Action of Hexamethonium

By Woo Choo Lee, Ph.D., M.D. and F. E. Shideman, Ph.D., M.D.

The positive inotropic action of hexamethonium in hypodynamic and freshly prepared papillary muscles of the cat and the hypodynamic heart-lung preparation of the dog has been demonstrated. Within certain limits, there exists a proportionality between the concentration of hexamethonium and the magnitude of the inotropic response. In both preparations results have been obtained which indicate that a state of partial refractoriness to the positive inotropic action of hexamethonium may be produced.

Following the demonstration of the ganglionic blocking activity of hexamethonium, an extensive investigation of its cardiovascular effects ensued. In spite of the fact that many facets of its action on the circulatory system have been elucidated, the direct effects, qualitative as well as quantitative, of this quaternary ammonium compound on myocardial contractility remain obscure. The experiments described below were performed to clarify this aspect of its pharmacology.

METHODS

Estimation of Hexamethonium in Plasma. Several micromethods for the determination of quaternary ammonium compounds in biological media have been described. The methyl orange method of Brodie and Udenfriend1 which is nonspecific and has been employed for the quantitative estimation of a number of organic bases has been found unsatisfactory for the determination of hexamethonium in serum. Mitchell and Clark3 were able to modify Auerbach's bromphenol blue method and determine hexamethonium in urine in concentrations as low as 2.5 ng./ml. When they applied this technic to undiluted plasma, quantitative recoveries were not realized. The major problem in the determination of hexamethonium in plasma appeared to be removal of the plasma proteins free of drug prior to addition of the dye. The method outlined below has proved satisfactory for precipitating the plasma proteins and extracting the drug, and has made possible the determination of the drug in concentrations as low as 3 μg./ml. in undiluted plasma.

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This investigation was supported in part by a grant from the Wisconsin Heart Association.

Received for publication September 10, 1957.

To the plasma is added half its volume of 5 per cent metaphosphoric acid. After standing for at least 1 hour, the mixture is placed in a boiling water bath for 5 min. in order to completely denature the proteins, then centrifuged for 20 min. at 2000 r.p.m. To 3 or 4 ml. of the clear supernatant fluid in a stoppered centrifuge tube are added 1.5 Gm. of sodium carbonate and 0.2 Gm. of dibasic potassium phosphate followed by 1 ml. of a solution of bromphenol blue (80 mg. per 100 ml. of 30 per cent K2HPO4; this reagent should be prepared each day). After the addition of 10 ml. of ethylene dichloride containing 3 per cent isoamyl alcohol, the tube is shaken for 20 to 30 min. in a mechanical shaker. The contents of the tube are centrifuged for 5 min. at 1500 r.p.m. and the optical density of the upper, ethylene dichloride, layer is determined by means of a Beckman model B spectrophotometer at a wavelength of 600 mμ. A standard curve relating concentration of hexamethonium to optical density is prepared each time samples of plasma containing unknown amounts of drug are analyzed. When plasma containing no drug is subjected to the same procedure the optical density of the ethylene dichloride extract is essentially no different than that of ethylene dichloride itself. When samples of plasma containing between 3 and 15 ng./ml. were analyzed by this method, recoveries ranging from 88 to 94 per cent were obtained. If the quantity of ethylene dichloride-isoamyl alcohol mixture is reduced to 5 ml., as little as 2 ng. of hexamethonium/ml. of plasma can be determined without a significant increase in error.

Papillary Muscle Preparations. The papillary muscle of the cat was prepared according to the procedure described by Cattell and Gold.* Cats were anesthetized with ether and killed by cardiotomy. One or two papillary muscles were carefully isolated from the right ventricle and placed in muscle chambers containing 100 ml. of a modified Tyrode's solution maintained at a constant temperature of 38 C. The medium employed has been described by Bennett (personal communication) and has the following composition in grams...
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per liter: NaCl, 7.000; KCl, 0.354; CaCl₂·2H₂O, 0.350; KH₂PO₄, 0.081; MgSO₄·7H₂O, 0.147; NaHCO₃, 2.100; Glucose, 0.900. A mixture of 95 per cent oxygen and 5 per cent carbon dioxide was bubbled through the bathing fluid by means of a sintered glass plate at the bottom of the muscle chamber. The muscles were stimulated to contract by means of a square wave stimulator which provided, at supra-maximal voltage, 1 impulse/sec. with a duration of 1 msec. Isotonic contractile amplitude was recorded on a smoked drum by means of a lever. Drugs were not added to the bath until the muscle had attained a constant amplitude of contraction.

Heart-Lung Preparation. Male and female dogs weighing 6 to 10 Kg. and anesthetized by the intravenous administration of sodium pentobarbital (30 mg./Kg.) were employed for these studies. The Krayer-Mendez* modification of the Patterson-Starling heart-lung preparation was used without the venous inflow pump and the coronary arterial cannula, and the Stolnikov stromuhr was used in place of the Weese type. The temperature of the blood entering the heart was maintained at 39 C. Right atrial pressure was continuously recorded with a water manometer and the systemic arterial pressure was followed by means of a mercury manometer connected to a side tube of the arterial cannula. The artificial resistance was set at 80 mm. Hg and the blood level in the venous reservoir was constantly maintained at 20 cm. above the opening of the superior vena cava. Periodic determinations of systemic cardiac output (left ventricular output minus coronary flow) were made. Venous inflow was regulated by means of a screw clamp on the venous inflow tube. The clamp was usually adjusted to result in a systemic cardiac output of 300 to 400 ml./min. In addition to these measurements, heart rate and competence index were determined before and after each administration of drug. The competence index, a measure of cardiac reserve, was calculated from the response of the pressure in the right atrium to an increase of 5 cm. in the height of the blood level in the venous reservoir. All drugs were administered, with mixing, by way of the venous reservoir.

RESULTS
A study of various concentrations of hexamethonium on the freshly prepared papillary muscle showed that a concentration of 100 µg./ml. had little or no effect. Three hundred µg./ml. exerts a slight positive inotropic effect, and larger doses, e.g., 1 mg./ml., cause a pronounced increase in the amplitude of contraction. The hypodynamic muscle is much more responsive to the positive inotropic action of the drug. After the addition of 100 to 150 µg./ml. of pentobarbital there was a marked decrease in contractile amplitude and subsequently 300 to 500 µg./ml. of hexamethonium resulted in a pronounced positive inotropic action.

The responses of 13 freshly prepared and 10 hypodynamic papillary muscles to hexamethonium were converted into percentage changes by comparing the contractile amplitude before and after the addition of the drug. These results are presented graphically in figure 1. The data on which the upper curve is based were obtained from muscles which had been rendered hypodynamic by addition of pentobarbital (100 µg./ml.) to the bath. It may be concluded that hexamethonium has a positive inotropic action at concentrations of 100 µg./ml. or more, in both freshly prepared and hypodynamic muscles, but the responses are more pronounced in the hypodynamic state. Furthermore, the degree of positive inotropic action can be correlated with the concentration of the drug.

Table 1 summarizes the responses to hexamethonium in 10 freshly prepared heart-lung preparations. It is to be noted that at the concentrations employed there were no significant changes observed in right atrial
TABLE 1.—Response of the Freshly Prepared Dog Heart-Lung Preparation to Various Concentrations of Hexamethonium

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Plasma concentration of hexamethonium (μg./ml.)</th>
<th>Right atrial pressure (mm. Hg)</th>
<th>Percentage increase or decrease in competence index</th>
<th>Systemic cardiac output</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before</td>
<td>After</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>11</td>
<td>18</td>
<td>18</td>
<td>+2</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>20</td>
<td>21</td>
<td>-4</td>
</tr>
<tr>
<td>3</td>
<td>16</td>
<td>19</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>23</td>
<td>11</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
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<td>23</td>
<td>6</td>
<td>3</td>
<td>0</td>
</tr>
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<td>35</td>
<td>3</td>
<td>3</td>
<td>+1</td>
</tr>
<tr>
<td>7</td>
<td>50</td>
<td>15</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>72</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>177</td>
<td>12</td>
<td>8</td>
<td>+2</td>
</tr>
<tr>
<td>10</td>
<td>200</td>
<td>16</td>
<td>14</td>
<td>+4</td>
</tr>
</tbody>
</table>

When failure was induced by pentobarbital or chloral hydrate, hexamethonium was found to produce a marked positive inotropic action. After adding 100 mg. of hexamethonium to the venous reservoir, a concentration of 200 μg./ml. of plasma was realized. The right atrial pressure decreased and approached a normal level and the systemic cardiac output and competence index increased markedly. The results of all experiments of this type are summarized in figure 2. The values are expressed in terms of percentage reversal of the effect induced by the agent used to produce failure. The mean values represent the average of 4 to 6 experiments for each range of concentrations in figure 2. Minimal but significant positive inotropic effects occur at plasma concentrations of 50 to 100 μg./ml. and a maximal response appears to occur when the concentration of hexamethonium in plasma is approximately 200 μg./ml. Larger doses of hexamethonium did not effect a significantly greater restoration of competence.

In an attempt to obtain a graded response to hexamethonium in the hypodynamic heart, it was noted that when a single effective dose of the drug was administered in increments at varying spaced intervals of time, the effects were relatively small as compared to the situation when the drug was administered as a single, undivided dose. This phenomenon is illustrated in figure 3 in 2 preparations which had been brought to failure to a comparable degree with pentobarbital. In the experiment on the left, 4 successive doses of 50 mg. of hexamethonium were administered at intervals of 15 min. The first dose produced a slight positive inotropic action as evidenced by the effect on right atrial pressure, competence index, and systemic cardiac output. Subsequent doses produced no further positive inotropic action. On the other hand, when the same total amount of drug was given in a single dose (experiment on the right), the response was much more marked even though a somewhat lower concentration of hexamethonium in plasma was realized than in the former experiment.

An observation similar to that described above has been made in the case of the hypodynamic papillary muscle of the cat. Two such muscles were obtained from the right ventricle of a single cat and stimulated simultaneously under like conditions in separate
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Fig. 3. The difference in the inotropic response of the hypodynamic heart-lung preparation of the dog to single and divided doses of hexamethonium (C). The values in parentheses below the kymographic records represent the concentrations of hexamethonium attained in the plasma after administration of the indicated doses. C.I., competence index; C.O., systemic cardiac output.

Moyer, et al. reported a decrease in cardiac output in the dog 30 min. after the administration of hexamethonium (5 mg./Kg.) and stated that this decrease occurred at a time when the calculated peripheral resistance had returned almost to the control level. Crumpton, Rowe, O’Brien and Murphy, utilizing cardiac catheterization for the measurement of cardiac output in the dog, also found that 30 min. after the administration of hexamethonium (1 mg./Kg.) there was a 31 per cent reduction in cardiac output and only a 13 per cent decrease in mean arterial blood pressure. Under these conditions the calculated total peripheral resistance was observed to increase. These results suggested to the authors that the reduction in mean arterial pressure was the result of a reduced cardiac output. Whether this was a consequence of a decrease in venous return or the result of a specific (negative inotropic) action on the myocardium was not determined. Paton and Zaimis could not demonstrate that hexamethonium produces a cardiac depressant effect in the cat, even in large doses. Wien and Mason showed that when a dose of hexamethonium as large as 20 to 40 mg. was injected into the side arm leading to the perfused aorta in the isolated heart of the cat (Langendorff preparation), there was an increase in amplitude of contraction; a smaller amount, such as 1 mg., was without effect. It is apparent from

muscle chambers. Quantitatively similar hypodynamic states were produced in both muscles by the addition of sufficient pentobarbital to the medium to result in a final concentration of 100 µg./ml. To the medium bathing one muscle was added 40 mg. of hexamethonium as a single dose and to the other the drug was added in increments of 5 mg. every 5 min. until the same total dose (40 mg.) was attained. Seven experiments of this type were performed. After the addition of 40 mg. as a single dose the mean contractile amplitude was increased 71.9 per cent (S.E. = ±4.3 per cent), whereas the comparable value obtained when hexamethonium was added in increments of 5 mg. was 37.8 per cent (S.E. = ±3.2 per cent). The difference between these two values is statistically significant (p < 0.02).

DISCUSSION

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the published information that the direct effects of hexamethonium on the heart are not clear. However, the results obtained in these experiments show that hexamethonium at concentrations of 50 to 100 µg./ml. or greater exerts a significant positive inotropic action. Lesser concentrations produce little or no effect. Therefore, it may be concluded that the decreases of cardiac output which have been observed after the administration of hexamethonium were not the result of a direct depressant action on the myocardium but to some other factor, or factors, perhaps the decreased venous return resulting from the peripheral vasodilation.

It is interesting to note that the administration of hexamethonium in increments of dosage does not produce as marked a positive inotropic action as does a single dose which is equal to the sum of the increments. It would appear that a state of refractoriness or tolerance may develop to the positive inotropic action of this compound on the myocardium. The production of tolerance to an effect of hexamethonium on another component of the cardiovascular system is suggested but not established by the work of Wien and Mason who observed the effects of this compound on coronary outflow in the anesthetized dog. No consistent change in outflow was effected by the drug, but when an alteration in coronary flow was realized it was not always possible to repeat it later in the experiment. Epinephrine, however, invariably resulted in reproducible increases in coronary flow.

**Summary**

A method for the estimation of hexamethonium in plasma has been described and the inotropic action of this drug has been studied in both the papillary muscle of the cat and the heart-lung preparation of the dog. At concentrations below 100 µg./ml., hexamethonium exerts no significant effect on contractility of either preparation. Concentrations higher than this produce a positive inotropic action in freshly prepared or hypodynamic papillary muscles and in the hypodynamic heart-lung preparation. The response, within certain limits, is proportional to the concentration of hexamethonium. This drug produces no significant inotropic action in the non-failed heart-lung preparation.

Both the hypodynamic papillary muscle and heart-lung preparation show a significantly greater response to a single effective dose of hexamethonium than when this same dose is administered in increments at spaced intervals of time. This observation is believed to indicate the development of a refractoriness by the myocardium to the positive inotropic action of hexamethonium.

**Summary in Interlingua**

Ez describite un metodo pro le estimation de hexamethonium in plasma. Le action inotropic de iste droga esseva studiate tanto in le musculo papillari del catto como etiam in le preparato de corde e pulmon del can. A concentrationes de infra 100 µg per ml, hexamethonium exerce nulle effecto significative super le contractilitate del duo preparatos. Concentrationes plus elevate produce un positive action inotropic in frescamente preparate o hypodynamic musculos papillari e in le hypodynamic preparato de corde e pulmon. Intra certe limites, le responsa es proportional al concentration de hexamethonium. Le droga non produce un significative action inotropic in non-hypodynamic preparatos de corde e pulmon.

Tanto le hypodynamic musculo papillari e le hypodynamic preparato de corde e pulmon exhibi un significativemente plus forte responsa a un sol dose efficace de hexamethonium que al administration del mesme dose in portiones intervallate. Iste observation pare indicar que le myocardio disveloppa refractorietate al positive action inotropic de hexamethonium.

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_Circ. Res._ 1958;6:66-71
doi: 10.1161/01.RES.6.1.66

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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