Mechanism of the Positive Inotropic Actions of Quaternary Ammonium Compounds

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ABSTRACT

The mechanism of the positive inotropic responses to quaternary ammonium compounds (tetraethylammonium, tetra-n-propylammonium, trimethyllethylammonium trimethyl-n-butylammonium, trimethyl-n-pentylammonium, trimethyl-n-hexylammonium, trimethylphenylammonium, trimethylbenzylammonium, triethylphenylammonium and m-hydroxyphenyltrimethylammonium) was examined on the atropinized papillary muscle of cats. After pretreatment with dichloroisoproterenol, all the quaternary ammonium compounds failed to produce their usual positive inotropic activities. Bretylium or TM-10, which specifically interferes with the release and/or synthesis of adrenergic mediators, rendered papillary muscle unresponsive to quaternary ammonium compounds but responsive to norepinephrine. Quaternary ammonium compounds also failed to produce their positive inotropic activity on papillary muscle whose catecholamines were almost completely depleted by treatment with reserpine. Surgical removal of the sympathetic innervation to the heart resulted in a marked reduction of myocardial catecholamines. The positive inotropic responses to quaternary ammonium compounds were markedly suppressed in papillary muscle obtained from bilaterally-sympathectomized cats with degenerated postganglionic sympathetic nerve fibers to the heart.

From the above results, it appears that quaternary ammonium compounds act at a common site to effect positive inotropic activities which are mediated via a catecholamine-release mechanism.

Since 1869, when Crum-Brown and Fraser (1868-1869) recognized that quaternary ammonium salts have curariform properties, these drugs have been a favorite subject of pharmacological investigation. They have been found to elicit a considerable variety of responses, some of which are now commonly associated with the pharmacology of these compounds, although the responses in question are by no means limited to the quaternary ammonium ion. Extensive studies have been reported concerning their various pharmacological activities in both animal and man. However, relatively few reports in the literature have described the cardiac actions other than the muscarinic responses of these compounds.

Acheson and Moe (1945), using the heart-lung preparation of the dog, found that tetraethylammonium exerted a positive inotropic action, as evidenced by a decrease in venous pressure and heart volume and an increase in systemic cardiac output. Since then, an occasional study has dealt with the cardiotonic activity of one or two of the quaternary ammonium compounds (Lee and Shideman, 1958). However, the positive inotropic activities of quaternary ammonium compounds were fully elucidated only as a result of the detailed work of Lee and

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The authors are indebted to the Burroughs Wellcome & Company, Inc. for providing bretylium tosylate (Daranthin); Smith Kline & French Laboratories for choline 2-6-nitrophenyl bicyclidine (TM-10); Eli Lilly & Company for dichloroisoproterenol (DCI); Ciba Pharmaceutical Products, Inc., for reserpine (Serpasil).
Shideman (1959b). Employing the isolated papillary muscle of the cat and the heart-lung preparation of the dog, they determined the relative cardiac activities of members of various series of quaternary ammonium compounds and suggested that positive inotropic action is a fundamental property of the quaternary ammonium ion. The present studies were undertaken to elucidate the mechanism of this universal positive inotropic action of quaternary ammonium compounds.

**METHOD AND MATERIALS**

The quaternay ammonium compounds studied were tetraethylammonium iodide, tetra-n-propylammonium iodide, trimethylammonium bromide, trimethyl-n-butylammonium bromide, trimethyl-n-pentylammonium bromide, trimethyl-n-hexylammonium bromide, trimethylphenylammonium iodide, trimethylbenzylammonium chloride, triethylphenylammonium iodide and m-hydroxyphenyltrimethylammonium chloride. These compounds were chosen in consideration of the differences in their chemical structures. Tetraethylammonium and tetra-n-propylammonium belong to tetraalkylammonium compounds (RnN⁺) and trimethylammonium, trimethyl-n-butylammonium, trimethylpentylammonium and trimethylhexylammonium are derivatives of trimethyalkylammonium compounds (CH₃)nN⁺R. The rest of the compounds were chosen because they contain phenyl radicals, with little change in their structures.

**Papillary Muscle Preparation:** The papillary muscle of the cat was prepared according to the procedure described by Cattell and Gold (1938). One or two papillary muscles were carefully isolated from the right ventricle and placed on muscle chambers containing 100 ml of Tyrode's solution maintained at a constant temperature of 38°C. Oxygen was bubbled through the bathing fluid via a sintered glass plate at the bottom of the chamber. The muscles were stimulated to contract by means of a square wave stimulator which provided, at supramaximal voltage, one impulse per second with a duration of 1 milli-second. Isotonic contractile amplitude was recorded on a smoked drum by means of a lever. Drugs were added to the bath after the muscle had attained a constant amplitude of contraction, and the magnitude of change of contractile amplitude was expressed as a percent change relative to the amplitude prior to the addition of drug.

**Bilateral Sympathectomy:** Under ether and pentobarbital sodium anesthesia, the stellate and thoracic sympathetic ganglia down to T₈ of one side were removed by the antero-lateral approach through the second intercostal space. Before closure of the intercostal gap the lungs were maximally inflated in order to minimize the pneumothorax. The operation was performed under strict aseptic conditions and penicillin was given intramuscularly following the operation. After an interval of 7-10 days had elapsed, the sympathetic ganglia of the other side were removed by the same procedure. The immediate effect of the operation was to cause constriction of the pupil and paralysis of the nictitating membrane. No other effects were observed and the animals were active until they were killed for the experiment. 15-20 days after the last operation.

**Determination of Catecholamines in Myocardium:** The catecholamine content of cardiac muscle was determined spectrophotofluorometrically by a modification (Lee and Shideman, 1959b) of the procedure described by Shore and Olin (1958).

**RESULTS**

1. **The positive inotropic action of quaternary ammonium compounds on the atropinized papillary muscle.**

Since it was found in a previous report (Lee and Shideman, 1959b) that the positive inotropic activity of quaternary ammonium compounds was obscured by the concomitant presence of muscarinic and nicotinic action, the effect of each compound was examined in the presence of atropine sulfate (0.015 mM). In a preliminary experiment it was shown that at the concentration of 0.015 mM, atropine sulfate had no effect by itself but completely blocked the muscarinic effect of acetylcholine on the papillary muscle of the cat heart. In table 1 are summarized the results of studies on the contractile amplitude of papillary muscle. Each value with its calculated
standard error represents the average of seven experiments. It will be noted that all the quaternary ammonium compounds examined exert significant positive inotropic responses on the atropinized papillary muscle preparations.

2. Effects of 1-(3, 4-dichlorophenyl)-2-isopropylaminoethanol hydrochloride (dichloroisoproterenol, DCI) on the positive inotropic action of the quaternary ammonium compounds.

Since Moran and Perkins (1958) showed that the cardiotonic effects produced by the injection of epinephrine, norepinephrine and isoproterenol or post-ganglionic cardiac sympathetic nerve stimulation were inhibited by DCI, numerous investigators have demonstrated that DCI can specifically block the cardiac adrenergic receptor (Lee and Shideman, 1959a; Nickerson and Chan, 1961). In order to determine whether or not adrenergic receptors are involved in the positive inotropic activities of the quaternary ammonium compounds, the influence of DCI on their cardiac actions was examined in the presence of atropine.

A study of the effect of various concentrations of DCI on atropinized papillary muscle showed that at a concentration of $10^{-5}$ M, there was a pronounced increase of contractile amplitude. After the contractile amplitude of the preparation had returned to the level existing prior to treatment of DCI, the addition of norepinephrine or epinephrine failed to elicit its usual cardiotonic activity. Similar experiments were conducted with the quaternary ammonium compounds on the preparations pretreated with DCI ($10^{-4}$ M). None of these compounds exerted their positive inotropic responses even at concentrations 5-10 times higher than those listed in Table 1.

3. Effects of bretylium tosylate on the positive inotropic action of the quaternary ammonium compounds.

Boura and Green (1969) recently reported that bretylium was able to block in cats and rabbits the effect of electrical excitation of sympathetic nerves to the heart, but did not block the effect produced by injection of norepinephrine. Their studies and those of others (Aviado and Dil, 1960; Szerb, 1961) have shown that the selective blocking of sympathetic nerves by this drug is due to its interference with the release of norepinephrine from the nerve endings. These findings suggest its usefulness as a tool for studying the role and interplay of the adrenergic mediators in the cardiotonistimulant actions of drugs. In an attempt to examine the nature of the positive inotropic responses to the quaternary ammonium compounds, experiments were conducted on papillary muscle pretreated with bretylium. At a concentration of $10^{-4}$ M, bretylium tosylate alone produced a pronounced positive inotropic response. After an elapse of 20-30 minutes, when the contractile amplitude had returned to the level existing prior to the addition of bretylium, the positive inotropic activity of each of the quaternary ammonium compounds was examined in the presence of atropine. All of these compounds failed to produce their expected positive inotropic response, but epinephrine or norepinephrine elicited their cardiotonistimulant response.

4. Effects of choline 2,6-xyyl ether bromide (TM-10) on the positive inotropic action of the quaternary ammonium compounds.

Since Exley (1957) showed that TM-10 interfered with the release and or synthesis of the adrenergic mediator, this agent has been employed as a tool to determine whether or not the observed cardiotonistimulant action of a drug was dependent on the adrenergic mediator (Lee and Shideman, 1959a). In an attempt to further examine the role of the adrenergic mediators in the positive inotropic responses to quaternary ammonium compounds, each of these compounds was added into the bath containing papillary muscles pretreated with TM-10 ($10^{-4}$ - $10^{-3}$ M). All the quaternary ammonium compounds examined failed to produce an increase in contractile amplitude, but epinephrine and norepinephrine exhibited their usual activity. These results are identical with those observed in preparations pretreated with bretylium.

5. Experiments on papillary muscle from cats treated with reserpine.

Recently it has been repeatedly demonstrated that reserpine depletes catecholamines from their storage. The capacity of this compound to deplete norepineph-
Table 1. The positive inotropic activities of quaternary ammonium compounds on the atropinized papillary muscle of cats

<table>
<thead>
<tr>
<th>Ammonium Compounds</th>
<th>Chemical Formula (RN⁺)</th>
<th>Concentration (mM)</th>
<th>% Increase of contractile amplitude of papillary muscle from Normal cats*</th>
<th>Sympathectomized cats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetraethyl</td>
<td>(C₄H₁₀)₄</td>
<td>1.0</td>
<td>40.4±2.7</td>
<td>9.1; 16.7; 0; 9.0</td>
</tr>
<tr>
<td>Tetra-n-propyl</td>
<td>(C₄H₉)₄</td>
<td>1.0</td>
<td>20.4±4.3</td>
<td>9.2; 0; 10.0</td>
</tr>
<tr>
<td>Trimethylmethlyl</td>
<td>(C₅H₁₃)₃·C₄H₅</td>
<td>1.0</td>
<td>8.1±0.01</td>
<td>0; 5.0</td>
</tr>
<tr>
<td>Trimethyl-n-butyl</td>
<td>(C₅H₁₃)₂·C₆H₁₂</td>
<td>1.0</td>
<td>35.0±3.3</td>
<td>12.0; 8.5; 10.0</td>
</tr>
<tr>
<td>Trimethyl-n-pentyl</td>
<td>(C₅H₁₃)₃·C₆H₁₂</td>
<td>1.0</td>
<td>50.0±4.0</td>
<td>20.0; 10.5; 8.0</td>
</tr>
<tr>
<td>Trimethyl-n-hexyl</td>
<td>(C₅H₁₃)₄·C₆H₁₂</td>
<td>1.0</td>
<td>83.8±9.2</td>
<td>12.5; 9.0; 15.5</td>
</tr>
<tr>
<td>Trimethylphenyl</td>
<td>(C₅H₁₃)₅·C₆H₁₆</td>
<td>0.2</td>
<td>38.8±4.2</td>
<td>8.5; 15.0; 13.5; 5.0</td>
</tr>
<tr>
<td>Trimethylbenzyl</td>
<td>(C₆H₅)₂·C₆H₁₂·C₆H₁₂</td>
<td>0.2</td>
<td>31.5±3.1</td>
<td>18.0; 5.0; 8.0</td>
</tr>
<tr>
<td>Triethylbenzyl</td>
<td>(C₆H₅)₃·C₆H₁₂·C₆H₁₂</td>
<td>0.2</td>
<td>39.5±3.8</td>
<td>12.0; 8.5; 9.5</td>
</tr>
<tr>
<td>m-hydroxyphenyltrimethyl</td>
<td>(C₆H₅)₂·C₆H₁₂·COH</td>
<td>0.2</td>
<td>49.7±5.2</td>
<td>28.0; 8.0; 15.0; 5.0</td>
</tr>
</tbody>
</table>

* Each value with its calculated standard error represents an average of 7 experiments.

Hraine and epinephrine from myocardium (Carlsson et al., 1957; Lee and Shideman, 1959a) has provided a potent tool for studying the role and interplay of these neurohormones on the cardiostimulant action of drugs. In an attempt to elucidate further the mechanism of the positive inotropic responses to quaternary ammonium compounds, experiments were performed on the papillary muscles whose catecholamines were depleted by the administration of reserpine. Reserpine was dissolved in a 1:1:2 mixture of ethyl alcohol, propylene glycol and water, and administered intraperitoneally in doses of 3 mg/kg. Twenty-four hours after the injection of reserpine, the cats were killed and their papillary muscles prepared as described previously and at the same time the catecholamine content of their myocardium was determined. As shown in table 2, such treatment with reserpine resulted in an almost complete depletion of myocardial catecholamines. The papillary muscle from these cats failed to elicit the positive inotropic response to each of the quaternary ammonium compounds in the presence of atropine.

Table 2. Myocardial catecholamine content of normal, reserpine-treated and bilaterally sympathectomized cats

<table>
<thead>
<tr>
<th>Myocardial Catecholamines (µg/g)</th>
<th>Normal</th>
<th>Reserpine-treated*</th>
<th>Bilateral sympathectomy†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.50</td>
<td>0.06</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>1.80</td>
<td>0.03</td>
<td>0.20</td>
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<td></td>
<td>1.65</td>
<td>0.08</td>
<td>0.25</td>
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<td></td>
<td>1.90</td>
<td>0.15</td>
<td>0.15</td>
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<tr>
<td></td>
<td>1.35</td>
<td>0.15</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>1.75</td>
<td>0.15</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>1.41</td>
<td>0.15</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>1.40</td>
<td>0.23</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>1.80</td>
<td>0.12</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>1.50</td>
<td>0.05</td>
<td>0.27</td>
</tr>
<tr>
<td>Mean ±S.E.</td>
<td>1.610 ±0.060</td>
<td>0.115±0.025</td>
<td>0.210±0.023</td>
</tr>
</tbody>
</table>

* Measured 24 hours after intravenous injection of 3 mg/kg of reserpine.
† Measured 15 to 20 days after bilateral sympathectomy.
‡ Significantly different from control value (P<0.001).

6. Experiments on the papillary muscle from bilaterally sympathectomized cats.

Numerous investigators have demonstrated that bilateral cardiac sympathectomy results in a marked reduction of myocardial catecholamines (Goodall and Kirshner, 1956; Cooper et al., 1961; Lee and Shideman, 1959). In order to further confirm the necessity of the presence of myocardial catecholamines in the positive inotropic responses to the quaternary ammonium compounds, experiments were performed on the papillary muscle from bilaterally sympathectomized cats with degenerated postganglionic sympathetic nerve fibers to the heart. Fifteen to 20 days
after the bilateral sympathectomy described previously, the cats were killed and their papillary muscles were tested with each of the quaternary ammonium compounds in the presence of atropine. The positive inotropic responses were markedly or almost completely suppressed in all the quaternary ammonium compounds examined (Table 1). The myocardial catecholamines of these sympathectomized cats were found to be decreased by approximately 87% (Table 2).

**DISCUSSION**

Considering the fact that all the quaternary ammonium compounds examined in this study exhibited qualitatively identical responses of the papillary muscles when treated with various drugs, it appears that these compounds act at a common site to effect a positive inotropic activity. The results of these experiments indicate that the positive inotropic responses to quaternary ammonium compounds are mediated via a release of catecholamines from myocardium. This finding agrees with those reported by several investigators who studied the mechanism of cardiac actions of one or two quaternary ammonium compounds. Thus, Lee and Shideman (1959a) demonstrated that tetramethylammonium (TMA), the parent and simplest quaternary ammonium compound, produced cardiotonic effects via release of catecholamines. Gilmore and Siegel (1962) reported that bretyllium, one of the quaternary ammonium compounds, elicits an immediate positive inotropic effect which is associated with a release of myocardial catecholamines. Hoffmann et al., (1945) and Lee and Shideman (1959a) studied the cardiotonic activity of acetylcholine and also concluded that such an action is due to the release of epinephrine-like substances.

Recent advances of various pharmacological tools for analyzing the cardiac adrenergic mechanism have made it possible to inquire into the mode of action of cardiotonic drugs. Dichloroisoproterenol, which specifically blocks the adrenergic receptors of the heart, prevented positive inotropic responses to quaternary ammonium compounds in doses capable of blocking the cardiotonic action of norepinephrine. This result indicates that adrenergic receptors of the heart participated in producing the positive inotropic activity of quaternary ammonium compounds. Furthermore, bretyllium or TM-10 which specifically prevent the release and/or synthesis of adrenergic mediators, rendered papillary muscle unresponsive to the positive inotropic activities of quaternary ammonium compounds but still allowed it to respond to exogenous epinephrine or norepinephrine. These findings provide evidence suggestive that epinephrine-like substances are implicated in the positive inotropic responses to quaternary ammonium compounds.

The emerging picture of reserpine as a depolarizer of norepinephrine and epinephrine from tissues furnished a useful tool for studying the role and interplay of these endogenous catecholamines in the cardiotonic activities. Thus, tyramine has been shown to act indirectly by releasing norepinephrine through the observation that its pressor action was markedly reduced in cats treated with reserpine (Carlsson et al., 1957) and the reduced action could be restored by an infusion of norepinephrine into the blood stream (Burn and Rand, 1958). Bejrablaya et al., (1958), studying the heart-rate effect in heart-lung preparations of normal and reserpine-treated dogs, concluded that sympathomimetic amines may be divided into two classes, one consisting of substances like norepinephrine which act directly on the adrenergic receptor, and the other consisting of tyramine and similar amines which act indirectly by releasing endogenous catecholamines.

We observed in this experiment that papillary muscle from cats whose myocardial catecholamines were almost completely depleted by treatment with reserpine failed to produce positive inotropic responses to quaternary ammonium compounds. This observation indicates that the presence of endogenous catecholamines is essential for the positive inotropic activities of quaternary ammonium compounds.

We also found that on papillary muscle from bilaterally sympathectomized cats the positive inotropic responses to quaternary ammonium compounds were markedly suppressed. This finding further supports
the above view that the positive inotropic activities of quaternary ammonium compounds are dependent on the presence of myocardial catecholamines, since bilateral sympathectomy resulted in a marked reduction of the catecholamine content. On the other hand, the result of the experiment on papillary muscle from bilaterally sympathectomized cats may be interpreted to indicate that the postganglionic sympathetic nerve endings in the heart are involved in the positive inotropic responses to quaternary ammonium compounds. However, further experiments are needed to determine the role of the sympathetic nerve endings in the mechanism by which quaternary ammonium compounds exert their positive inotropic effect. Lee et al (1960), employing noninnervated heart of the 4-day old chick embryo, demonstrated that the cardiotonic effects of TMA, acetylcholine and nicotine are not dependent on the presence of sympathetic nerve endings in the heart. They hypothesized certain structures between the adrenergic receptors of the myocardium and the postganglionic sympathetic nerve endings upon which TMA or acetylcholine act to release epinephrine-like substances. Moe and Freyburger (1960) reported that the cardiotonic activity of tetraethylammonium could be demonstrated in the embryonic chick heart before nerves reach the cardiac tissue. Considering the close similarity between the positive inotropic responses to TMA studied by Lee et al. (1960) and the positive inotropic responses to the quaternary ammonium compounds studied in this experiment, it appears that the same site as that proposed by Lee et al. (1960) is involved in a release of catecholamines by the quaternary ammonium compounds.

REFERENCES

