Pharmacological Evidence for the Importance of Catecholamines in Cardiac Rhythm

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Electrophysiological investigations have related intrinsic rhythmicity of the specialized tissues of the heart to slow diastolic depolarization, although attempts to elucidate it further have not been successful.1 Hoffman and Cranefield1 suggest the possibility that the slow depolarization is due to a change in the net membrane current and thus to a change in permeability to one or more ions. Several investigators2-4 have argued that intrinsic rhythmicity stems directly from acetylcholine synthesis, an assumption which is supported by the fact that the sinus venosus of the frog heart differs from atrial and ventricular muscle in its higher content of acetylcholine cholinesterase, cholineacetylase and Na+ ion, and lower content of K+ ion.5 Using the heart-lung preparation, Krayer and his co-workers6-8 reported that norepinephrine stores in cardiac tissue are involved in maintaining sinus activity. Since catecholamines increase the rate of diastolic depolarization, it is possible that this furnishes evidence for a relationship between catecholamine activity and intrinsic rhythmicity of pacemaker sites.

The present work is concerned with the contribution of catecholamines to the rhythmicity of pacemaker sites in dogs with surgically induced complete heart block. This preparation was used because it makes it possible to record simultaneously the independent responses of atrial and ventricular pacemakers. The experiments indicated that the so-called inherent rhythmicity of the ventricle is more dependent on catecholamine activity than is that of the atrium.

Methods

Heart block was induced by severing or ligating the bundle of His 5 to 10 mm. anterior to the coronary sinus along the axis of the A-V junctional area, according to the methods of Starzl, Gaertner9 and Taufic et al.10 The operations were performed with aseptic precautions on dogs weighing between 10 and 20 Kg. which were anesthetized with 30 mg. per Kg. of pentobarbital given intravenously. Oxygen (100 per cent) was administered throughout the operation through a well-fixed endotracheal tube connected to a demand pneumoflator.11 Electrocardiograms were used to confirm the development of A-V dissociation. In some cases, the sinus resumed control after a brief period of A-V dissociation; in these, the operative procedure was repeated, but occasionally, despite numerous attempts, permanent heart block could not be induced. In all cases, NT-methylatropine sulfate, 200 /µg. per Kg., was administered to test whether the block was of vagal origin.

During the procedure, the animals were infused with normal saline or 5 per cent dextrose solution. If there was excessive blood loss, the estimated loss was replaced by blood from a donor dog. Postoperatively, the dogs were given 400,000 units penicillin and 1 Gm. dihydrostreptomycin for at least three days.

Adrenalectomy was performed on four animals. These animals were treated daily with cortisone acetate (12.5 to 25 mg.) intramuscularly. Blood pH and electrolytes were determined before and after adrenalectomy. Sympathectomy was performed in three of these animals by removing the thoracic sympathetic chain bilaterally.

The influence of sympathomimetic activity on pacemaker rhythmicity was also explored through the use of hexamethonium chloride (C6), reserpine, and N-methylatropine sulfate. In the experimental procedures, all drugs were administered rapidly and intravenously. The concentrations were ad-
Table 1

Summary of Results

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of dogs</th>
<th>Dose (mg./Kg.)</th>
<th>Average atrial rate beats/min. ± S.E.*</th>
<th>Average ventricular rate beats/min. ± S.E.*</th>
<th>AV/Ratio ± S.E.*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20</td>
<td></td>
<td>156 ± 5.4</td>
<td>46 ± 3.6</td>
<td>3.8 ± 0.3</td>
</tr>
<tr>
<td>N-methylatropine</td>
<td>16</td>
<td>0.2</td>
<td>185 ± 6.0</td>
<td>48 ± 4.5</td>
<td>4.3 ± 0.3</td>
</tr>
<tr>
<td>Hexamethonium</td>
<td>16</td>
<td>2.0</td>
<td>144 ± 5.6</td>
<td>24 ± 3.0</td>
<td>6.0 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4.0</td>
<td>139 ± 13.7</td>
<td>22 ± 4.5</td>
<td>7.8 ± 2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>8.0</td>
<td>145 ± 3.8</td>
<td>20 ± 3.8</td>
<td>8.0 ± 1.3</td>
</tr>
<tr>
<td>After reserpine</td>
<td>14</td>
<td>0.1</td>
<td>123 ± 4.6</td>
<td>25 ± 2.2</td>
<td>5.4 ± 0.4</td>
</tr>
<tr>
<td>And N-methylatropine</td>
<td>7</td>
<td>0.2</td>
<td>133 ± 7.4</td>
<td>24 ± 2.5</td>
<td>6.0 ± 0.6</td>
</tr>
<tr>
<td>And hexamethonium</td>
<td>9</td>
<td>2.0</td>
<td>126 ± 5.4</td>
<td>14 ± 1.5</td>
<td>9.9 ± 1</td>
</tr>
<tr>
<td>Control§</td>
<td>4</td>
<td></td>
<td>131 ± 7.2</td>
<td>43 ± 5.4</td>
<td>3.1 ± 0.3</td>
</tr>
<tr>
<td>After adrenalectomy</td>
<td>4</td>
<td></td>
<td>120 ± 9.0</td>
<td>38 ± 4.8</td>
<td>3.6 ± 0.6</td>
</tr>
<tr>
<td>And sympathectomy</td>
<td>3</td>
<td></td>
<td>87 ± 4.1</td>
<td>17 ± 1.2</td>
<td>5.5 ± 0.4</td>
</tr>
</tbody>
</table>

*S. E. = standard error.
†Atrial rate
‡Ventricular rate
§Two animals died 24 and 32 hours after reserpine.

N-methylatropine was not used in this group.

In the animals with heart block, N-methylatropine did not affect the ventricular rate (table 1), but, as expected, it accelerated the atrial rate. N-methylatropine did not change the QRS configuration or QRS time.

The electrocardiograms were recorded on a Sanborn polygraph, using leads I and II. Control records were made for at least 30 minutes before a drug was administered. A 20-second record was taken to represent the heart rate for any observation.

Results

Effect of Hexamethonium (Co)

Since Co blocks transmission in both sympathetic and parasympathetic ganglia, acceleration of the atrial rate may develop after the drug if vagal influence exceeds that of the sympathetic nervous system. However, since N-methylatropine was given 10 to 15 minutes prior to the administration of Co (fig. 1), this was not a factor in these experiments. It may be seen from table 1 and figure 1 that Co reduced both the atrial and ventricular rates, the peak effect developing 10 to 20 minutes after injection. The ventricular rate was slowed more than the atrial rate; as a result, the A/V* ratio rose from 4.3 to 6.9 (table 1). Larger doses of Co (4 and 8 mg.

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justed so that 0.1 cc. per Kg. was injected in each instance. Except for N-methylatropine, all doses are expressed in terms of the free base.

In the reserpinized dog, a bloody diarrhea sometimes provided a special problem. In these animals, the blood was tested periodically for disturbances in electrolyte content, pH, and CO₂ combining power and, where necessary, the indicated measures were instituted.

After the dogs had recovered from the operative procedure, they were trained for a period, usually five to seven days, to lie quietly during injections and the recording of electrocardiograms. In these animals, ventricular rates remained remarkably constant from day to day and did not vary spontaneously. Equally striking was the small variation in ventricular rates between animals as indicated by the standard error (table 1). Atrial rates tended to show cyclic variations. In one case, for example, during a 30-minute control period, there was a variation of 40 beats per minute, which was cyclic and sometimes appeared to be related to respiration.

Since N-methylatropine abolished the cyclic variation in sinus rates, indicating that the variations were related to vagal tone, in order to determine the direct effect on the sinus, drugs were given after the administration of N-methylatropine. The 200 μg. per Kg. dose of N-methylatropine produced complete vagal blockade for at least two and a half hours. This was verified by stimulating the vagus nerve of normal dogs anesthetized with intravenous pentobarbital, and recording the effect on the sinus rate before and after N-methylatropine.
INTRINSIC CARDIAC RHYTHMICITY

per Kg.) did not produce effects significantly different from those after the smaller dose (table 1).

In these experiments, the idioventricular rate cannot always be used as a measure of the effect of Co on a particular pacemaker site in the ventricle, since after Co the QRS configuration and the QRS time sometimes changed, suggesting a shift in the pacemaker site (fig. 1).

In an adrenalectomized animal, Co produced transient ventricular asystole, although the atrium continued to beat at a rate of 126 per minute.

**Effect of Reserpine**

Reserpine produced effects similar to those of Co, the A/V ratio rising (table 1) from 4.3 to 5.4. It required 24 to 35 hours for peak effects to develop (fig. 2). In two dogs observed for seven days after reserpine, the heart rates had not returned to control levels; the period of recovery is, therefore, at least one week long.

It may be seen from table 1 and figure 1 that N-methylatropine given at the time of the peak effect of reserpine did not alter the atrial or ventricular rates. A vagal mechanism, therefore, seems to play no role in the depression of these rates by reserpine. Changes in QRS time and QRS configuration were often seen after reserpine, and in some instances as many as four distinctly different QRS complexes were observed in the same strip of tracing. Here too, therefore, the idioventricular rate cannot be used as a precise measure of depression of a particular pacemaker.

Larger doses of reserpine (up to 0.3 mg./Kg.) did not cause greater reductions of heart rate. However, a high fatality rate limited the observations on the larger doses. In this series of four animals, two dogs died after two doses of reserpine (each 0.1 mg. per Kg.) given on successive days; two others survived three such daily doses. Even in the case of the single dose of 0.1 mg. per Kg. of reserpine, two of the 14 dogs so treated died within a day of the injection. The cause of death was probably heart failure; postmortem examination revealed an enlarged heart, ascites, and pulmonary edema.

In an attempt to re-establish the control idioventricular rate, an infusion of norepinephrine was given to two dogs pretreated with a dose of 0.1 mg. per Kg. of reserpine. While no attempt was made to determine the precise amount of norepinephrine required, an infusion of 1 µg. per Kg. per minute, given over a period of 10 to 30 minutes, accelerated the pacemaker to approximately control rates. The effect of norepinephrine was not permanent, however, and waned soon after the infusion was terminated.

Boyer and Chisholm\(^1\) have shown that in animals with heart block, changes in blood electrolytes or pH alter both atrial and ventricular rates, and since bloody diarrhea sometimes developed during the course of the reserpine action, it was necessary to explore the possibility that the changes in the heart rate might, in part, be due to changes in electrolyte balance and pH. In three dogs treated with 0.1 mg. per Kg. of reserpine, blood electrolytes, pH, and CO\(_2\) combining power were determined. The results were not grossly abnormal.

**Combined Effect of Hexamethonium and Reserpine**

While it was clear from the foregoing results that ceiling effects were produced by the doses of 2 mg. per Kg. of Co, and 0.1 mg. per Kg. of reserpine, it was thought that the two agents in combination might induce more intense effects than either alone. This proved to be the case; Co administered at the peak of reserpine action further slowed the ventricle (table 1 and fig. 2). It is noteworthy that in this circumstance, the sinus rate was not further depressed (which suggests that ventricular rhythmicity is more dependent on sympathetic activity than that of the sinus).

**Effects of Adrenalectomy and Sympathectomy**

Adrenalectomy did not significantly alter the atrial and ventricular rates, whereas subsequent sympathectomy did (table 1). Since the sympathectomized animals did not survive long enough to ensure exhaustion of catecholamines, a precise quantitative com-
Discussion

While Waud et al. have described the importance of catecholamines to the rhythmicity of the sinus pacemaker, the present experiments show that catecholamine activity plays an even greater role in the rhythmicity of the ventricular pacemaker. Reserpine and C6 each always produced a greater reduction of ventricular than sinus rates; after adrenalectomy, C6 produced ventricular asystole, although only moderately slowing the atrial rate; given after reserpine, C6 reduced the ventricular rate, but it did not also slow the sinus rate. The reduction of ventricular rate by sympatheticotomy is not qualitatively different from that produced by the combined actions of reserpine and C6. This suggests that the state brought about by the combination of the drugs is comparable to that produced by surgical abolition of sympathetic activity. It required at least 24 hours for the peak effect of reserpine to develop, a curve of action which corresponds with the established course of catecholamine depletion by this drug. The evidence of Sheppard et al. that there was no correlation between the concentration of reserpine labeled with C14 or H3 and the intensity of a variety of reserpine effects further supports the argument that a direct action of reserpine on pacemaker tissue is not likely, whereas the effect on catecholamine activity is the more probable reason for cardiac slowing.

A dosage-response relationship seems to exist between rhythmicity and catecholamine activity; thus, after reserpine, C6 lowers the
ventricular rate below that produced by either agent alone, suggesting that a more nearly complete blockade of sympathetic activity may account for the greater reduction in cardiac rhythmicity. Since the combined action of C₆ and reserpine was greater than that of either alone, it seems clear that neither is capable of complete abolition of catecholamine activity. These results are consonant with those which show that large doses of C₆ do not always produce complete blockade of ganglionic transmission,¹⁴ and with those which show that depletion of stored catecholamines is not necessarily complete after reserpine.¹⁵,¹⁶

The similarity in the effects of C₆ and reserpine raises the question of how their actions may be related; it is possible, however, that although both reduce the availability of catecholamines, the sites of action differ. Catecholamines stored in the heart are probably liberated by sympathetic stimulation, a suggestion consistent with the finding that the effectiveness of the cardiac accelerator nerve is sharply reduced after reserpine.¹⁷

Experiences with isolated heart and isolated atrium preparations show that C₆ does not affect heart rate or force of contraction.¹⁸,¹⁹ In the heart-lung preparation, C₆ does not influence the reserpine release of catecholamines.⁸ It has been indicated that catecholamine depletion by reserpine decreases the sinus rate independently of the sympathetic nervous system.²⁰,²¹ The rate-decreasing effect of reserpine on the ventricle is also likely to be due to depletion of catecholamines. Therefore, although both C₆ and reserpine affect stored catecholamines, the action of reserpine seems to be one which liberates, and thereby exhausts the stores, while that of C₆ is through blockade of the sympathetic activity which normally liberates the amines.

Although catecholamine activity clearly plays an important role in the intrinsic rhythmicity of the ventricular pacemaker, since reduction in catecholamine activity by the measures employed in these experiments rarely produced ventricular asystole, there must be still another essential factor in its initiation and maintenance. Harris and co-workers²²,²³ indicated that an ectopic focus which develops after coronary occlusion may be rendered hyperexcitable, not only by the release of catecholamines from the necrotic cells, but also by the release of histamine and potassium. In addition, Maling et al.²⁴ reported that, after coronary occlusion, the development of ventricular arrhythmias is not affected by pretreatment with reserpine. Inasmuch as vagal blockade may reduce or abolish ventricular arrhythmias following coronary ligation,²⁵ their results would have been more definitive had they also evaluated the contribution of the vagal tone to the genesis of ventricular arrhythmia in the reserpinized dog.

Since reserpine produces bloody diarrhea, there was the possibility that effects on the heart, such as those observed after reserpine, could result from disturbances in pH and electrolyte due to the diarrhea. Our dogs were always treated to prevent such changes, and consequently, none were observed.

Acute hypotension by itself has been shown not to cause changes in cardiac excitability,²⁶ but it was possible that the extensive periods of low blood pressure after reserpine might indirectly have caused changes in heart rate. Prolonged hypotension is an unlikely factor in these experiments, since C₆, which also produces hypotension, induced decreases in heart rate of the same order as those after reserpine as promptly as 10 minutes after injection.

**Summary**

Dogs with complete heart block were used to explore the dependence of the intrinsic rhythmicity of the heart on catecholamine activity. By the use of reserpine, hexamethonium, and surgical sympathectomy, it was demonstrated that, while reduction in catecholamine activity slows both the atrial and the ventricular rates, the ventricular pacemaker was always affected to a greater degree. The effect of a combination of reserpine and hexamethonium on the ventricle was greater than when either agent was administered alone, suggesting that the greater the sympathetic blockade, the greater the depres-
sion in pacemaker rhythmicity. Since the course of its action on the heart paralleled that of catecholamine depletion, it seems probable that the effect of reserpine on rhythmicity was due to its catecholamine-depleting action. It was thought unlikely that prolonged hypotension contributed to the reserpine action on the heart. It is indicated that disturbances in electrolyte balance and blood pH did not play a role in the effects observed.

References
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