Concentration of Myocardial Cyclic AMP and Ventricular Fibrillation Induced by Aminophylline

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SUMMARY

The concentration of myocardial cyclic AMP was measured in 9 dogs by radioimmunoassay after the administration of aminophylline. Fourteen dogs served as control. The concentration of cyclic AMP in the left ventricle was the highest and the lowest value was obtained in the right atrium in the control dogs. Ventricular fibrillations were induced immediately after the injection of 30 mg/Kg aminophylline in 3 dogs out of 9. The concentration of the left ventricular cyclic AMP in 6 dogs which tolerated aminophylline was significantly elevated compared with that of the control dogs (p<0.05). The left ventricular cyclic AMP in 3 dogs with ventricular fibrillation was significantly higher compared with that in the aminophylline tolerated dogs with non-fibrillating hearts (p<0.01).

These results showed that the concentration of cyclic AMP was elevated in the fibrillating heart.

Additional Indexing Words:
Cyclic AMP Ventricular fibrillation Aminophylline

In a series of investigations, we measured the level of myocardial cyclic-AMP of dogs after the intravenous administration of aminophylline, an inhibitor of phosphodiesterase and it happened that ventricular fibrillation was induced in some of the experimental animals soon after injection of aminophylline. In this paper, the concentration of myocardial cyclic AMP of dogs with ventricular fibrillation induced by aminophylline is reported.

MATERIALS AND METHODS

Twenty-three mongrel dogs, weighing 5.0–7.0 Kg, were divided into 8 normal control dogs, 6 sham-operated dogs, and 9 experimental dogs, anesthetized with sodium hexobarbital and thoracotomized under artificial respiration. The heart

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Received for publication June 30, 1978.
was exposed and Courmand catheter was inserted into the left ventricular cavity to record the left ventricular pressure and the dp/dt with an electronic manometer (Nihon-Koden MP-3A). The cardiac muscle of 100–300 mg was frozen and torn out with tongs of stainless steel precooled in liquid nitrogen, when the dp/dt reached the maximum after 30 mg/Kg of aminophylline was administered from the right femoral vein.

Cyclic AMP concentration was measured with radioimmunoassay of Steiner modified by Okabayashi. The cardiac muscle was homogenized with 1 ml of 0.05 M sodium acetate buffer (pH=6.2). After centrifugation at 3000 rpm for 10 min, 50 µl of supernatant solution, 10 µl of 0.05 M sodium acetate buffer (pH=6.2), 10 µl of 0.5% bovine albumin, 10 µl of 0.2 pmol ³H-cyclic AMP, and 10 µl of cyclic AMP antibody were mixed and then reacted in the ice cold water for 1 hour. Fifty µl of reaction product was filtered through a Millipore membrane filter paper placed on a pyrex microanalysis filter holder and was washed by 5 ml portions of 0.05 M sodium acetate buffer for 3 times. The Millipore membrane filter paper on which ³H-cyclic AMP-cyclic AMP antibody complex was absorbed, was put into a mixture of 10 ml of Bray’s solution and 0.5 ml of H₂O in a counting vial and was counted in a liquid scintillation counter (Aloka 100) for 5 min. The standard radioimmunoassay curve indicated that the lowest amount of cyclic AMP measurable by this method was 0.5 pmol. In the cardiac muscle weighing 100 to 300 mg, the level of cyclic AMP had a linear correlation with muscle weight and was expressed as pmol/mg of wet weight of cardiac muscle.

Adenosin-³H (G) 3', 5'-cyclic monophosphate ammonium salt was obtained from New England Nuclear Corporation. Adenosine 3’, 5’-cyclic AMP was from Boehringer. Millipore membrane filter (H. A. pore size 0.45) was from Millipore corporation. Antibody to cyclic AMP was a gift from Dr. Okabayashi. Animophylline was obtained from Eisai Pharm Co. Other chemicals were obtained from commercial sources.

Results were analyzed for statistical significance by Student’s “t” test.

RESULTS

The concentration of cyclic AMP in the heart of normal dogs:

Table I shows the mean concentration of cyclic AMP in the heart of 8 dogs. The concentration in the left ventricle was the highest and the lowest was in the right atrium.

<table>
<thead>
<tr>
<th>Cardiac Muscle</th>
<th>No. of Experiments</th>
<th>Cyclic AMP (pmol/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left Ventricle</td>
<td>8</td>
<td>0.318±0.022</td>
</tr>
<tr>
<td>Right Ventricle</td>
<td>8</td>
<td>0.204±0.024</td>
</tr>
<tr>
<td>Left Atrium</td>
<td>8</td>
<td>0.131±0.031</td>
</tr>
<tr>
<td>Right Atrium</td>
<td>8</td>
<td>0.094±0.025</td>
</tr>
</tbody>
</table>

Mean ± S. E. M.
The concentration of cyclic AMP in the dog with aminophylline-induced ventricular fibrillation:

Ventricular fibrillations were induced within 5 min after the injection of aminophylline in 3 dogs although the other 6 developed no arrhythmia and survived after the treatment. The mean cardiac rate of 164.0±15.3/min at the beginning of the injection increased to 202.1±17.0/min after the injection of aminophylline. The mean dp/dt increased significantly by 160.3±17.8% after the injection (p<0.05).

As shown in Fig. 1, when dp/dt reached the maximum after the injection of 30 mg/Kg of aminophylline, the mean concentration of the left ventricular cyclic AMP of the dogs survived after aminophylline was 0.496±0.039 pmol/mg. This was a significant increase compared with the value of 0.315±0.047 pmol/mg in the 6 sham operated controls with p of less than 0.05. The right ventricular cyclic AMP of these 6 aminophylline-treated dogs was 0.428±0.027 pmol/mg and was also higher than that in the sham operated controls, 0.249±0.024 pmol/mg.

The mean concentration of the left ventricular cyclic AMP in dogs with aminophylline-induced ventricular fibrillation was 0.835±0.035 pmol/mg and this was significantly elevated when compared with that in the aminophylline-treated but survived dogs (p<0.01). The mean concentration of the right ventricular cyclic AMP in dogs with ventricular fibrillation was 0.591±0.051 pmol/mg and was also significantly increased when compared with dogs which survived after aminophylline (p<0.05).
DISCUSSION

In the present study, the concentration of the left ventricular cyclic AMP and the max dp/dt were increased after the injection of aminophylline. It was interesting that ventricular fibrillation was induced in some of the dogs soon after the administration of aminophylline and the concentration of the myocardial cyclic AMP in these dogs was significantly higher than that in the animals which survived after aminophylline. Podzuweit et al\(^3\) proposed that the development of ventricular fibrillation in ischemic heart disease and myocardial infarction could be related to accumulation of cyclic AMP in the ischemic zone. Horowitz et al\(^4\) demonstrated that aminophylline exerted a biphasic effects on the ventricular myocardium with regard to vulnerability to fibrillation. Within 5 min after the start of infusion, the ventricular fibrillation threshold was decreased by 30 to 35\%, the earliest measurable decrease occurring 4 min after the onset of infusion. During the early phase of infusion of aminophylline, ventricular fibrillation could be induced more easily than before the infusion. After the completion of intravenous infusion, aminophylline exerts a protective effects on the myocardium by reducing the ease with which fibrillation can be initiated. The mechanism by which aminophylline produces the effects on ventricular fibrillation threshold is speculative. The effects of epinephrine on ventricular fibrillation threshold closely parallel that of aminophylline. Han et al\(^5\) in explaining the biphasic effects of epinephrine postulated that this agents, when administered intravenously, was initially distributed nonuniformly within the myocardium. A similar nonuniform distribution of aminophylline within the myocardium would be expected to enhance disparity between the time course of recovery of neighboring ventricular fibers. This increased temporal dispersion of recovery predisposes to reentry and fractionation of depolarization wave fronts, thereby facilitating the initiation of ventricular fibrillation.\(^6\)

The posisitve inotropic response of aminophylline, an inhibitor of phosphodiesterase, is similar to that of catecholamine. Its effects on contractility are mimicked by exposure of myocardium to derivatives of cyclic AMP such as dibutryl cyclic AMP which resist degradation by phosphodiesterase. Lubbe et al\(^7\) reported that dibutryl cyclic 3', 5'-AMP enhanced ventricular vulnerability to fibrillation by lowering the ventricular fibrillation threshold and increasing the duration of the vulnerable periods. Ischemia,\(^8\) catecholamine,\(^9\) and aminophylline\(^4\) reduced the ventricular fibrillation threshold and are thought to provoke an intracellular accumulation of cyclic AMP. Mori\(^10\) reported that adenyl cyclase activity in the infarcted area was significantly increased when compared with the non-infarcted area at 15 min and this
tendency continued 60 min. On the other hand, phosphodiesterase activity in the infarcted area did not significantly changed. Nakamura et al\(^{20}\) reported that the level of C-GMP in the infarcted area of dog myocardium at 15 min was significantly higher than in the sham operated control and the level of cyclic AMP in both infarcted and non-infarcted area at 60 min was significantly higher than in the control dog myocardium. It has been postulated that the electrophysiological effects of cyclic AMP could facilitate slow channels in ischemic tissue,\(^3\) thereby facilitating reentry pathway and spontaneous self-exitation with the development of ventricular fibrillation.\(^{12}\) Although, there are some difficulties in the interpretation of experiments performed with phosphodiesterase inhibitors,\(^{12}-^{17}\) our findings suggest that there was a significant, close association between high concentration of myocardial cyclic AMP and ventricular fibrillation.

REFERENCES

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