Myocardial Norepinephrine and Cyclic AMP Concentration following Myocardial Ischemia — Relation to Ventricular Fibrillation and Sudden Death —

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This study was designed to investigate the relationships of myocardial concentrations of norepinephrine (NE) and cyclic AMP (c-AMP) to the development of ventricular fibrillation (VF) with reference to the effects of a premedication of dibutryl cyclic AMP (DBC-AMP) and propranolol in dogs with experimental myocardial infarction. Myocardial specimens were obtained serially from the ischemic and the non-ischemic zones by mini-drill biopsy, and NE and c-AMP levels were determined by high-performance liquid chromatography and radioimmunoassay, respectively. Before the occurrence of VF, myocardial NE increased in both the ischemic and the non-ischemic zones, and c-AMP increased significantly in the ischemic zone but did not in the non-ischemic zone. In dogs premedicated with DBC-AMP an increase of c-AMP was observed in both the ischemic and the non-ischemic zones in association with an increased incidence of VF.

On the other hand, no significant increase of myocardial c-AMP was observed in both the ischemic and the non-ischemic zones of propranolol-premedicated dogs which were free from VF. A significant increase of myocardial c-AMP in the ischemic zone was observed in dogs which suffered from VF in spite of the premedication of propranolol. The incidence of VF was significantly reduced by 26.5% in dogs pretreated with propranolol. No significant changes in myocardial norepinephrine and c-AMP were observed in dogs which were free from VF throughout the experiments.

It is well known that sudden death occurring in the early stage of acute myocardial infarction is mainly due to ventricular fibrillation (VF), and with the occurrence of myocardial infarction a compensatory hyperactivity of the adrenergic nervous system takes place.1,2 Plasma and urinary levels of norepinephrine and plasma cyclic AMP (c-AMP) elevate following acute myocardial infarction.3,4 On the other hand, there have been reports that the concentration of c-AMP in the ischemic myocardium rises before the onset of VF.5,6 Now the cardiac action of catecholamines is mediated by c-AMP. Thus, the present study was designed to clarify the relationships of the myocardial tissue concentrations of c-AMP and norepinephrine to the incidence of VF using dogs with experimentally induced myocardial infarction. The pharmacological effects of exogenously administered dibutyryl cyclic AMP (DBC-AMP) and propranolol on these relations were also studied.

Key Words:
Ventricular fibrillation
C-AMP
Norepinephrine
Propranolol
Myocardial ischemia

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TABLE I THE INCIDENCE OF VENTRICULAR FIBRILLATION (VF) IN VARIOUS EXPERIMENTAL MODELS

<table>
<thead>
<tr>
<th>Experimental Models</th>
<th>VF (+)</th>
<th>VF (−)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Ligation only</td>
<td>2 (20.0%)</td>
<td>8 (80.0%)</td>
</tr>
<tr>
<td>2 Ligation + Punching Biopsy</td>
<td>52 (50.0%)</td>
<td>52 (50.0%)</td>
</tr>
<tr>
<td>3 Ligation + Punching Biopsy + DBC-AMP</td>
<td>10 (55.6%)</td>
<td>8 (44.4%)</td>
</tr>
<tr>
<td>4 Ligation + DBC-AMP</td>
<td>7 (53.8%)</td>
<td>6 (46.2%)</td>
</tr>
<tr>
<td>5 Ligation + Punching Biopsy + Propranolol</td>
<td>6 (23.1%)</td>
<td>20 (76.9%)</td>
</tr>
</tbody>
</table>

*p < 0.05 and **p < 0.01 using χ²-test.

MATERIALS AND METHODS

This study was conducted on healthy adult mongrel dogs of both sexes ranging in weight between 15 and 25 kg. The animals were anesthetized with sodium pentobarbital (30 mg/kg) given intravenously. The trachea was intubated and artificial ventilation was instituted using a Harvard ventilator with room air. The lead III electrocardiogram was monitored continuously using a Sanei 2304-A Oscillographic Recorder throughout the experiment. Thoracotomy was performed at the left fourth intercostal space, and the heart was exposed following pericardiotomy. The left anterior descending coronary artery (LAD) was dissected free just distal to the circumflex artery, and a silk ligature was placed loosely around the vessel.

The dogs were divided into 5 groups: 1) Ligation-only group: 10 dogs with only LAD ligation. 2) Ligation plus punching biopsy group: 111 dogs with the LAD ligation and punching biopsy. 3) Ligation plus punching biopsy plus DBC-AMP group: 18 dogs received 10 mg/kg of DBC-AMP 20 min before the LAD ligation and punching biopsy. 4) Ligation plus DBC-AMP group: 13 dogs received 10 mg/kg of DBC-AMP 20 min before the LAD ligation. 5) Ligation plus punching biopsy plus propranolol group: 26 dogs received 0.5 mg/kg of propranolol 15 min before the LAD ligation. After a 30-min stabilization period, tiny myocardial samples from the left ventricle were taken by high speed mini-drill biopsy. After 30 sec, the LAD was ligated and punching biopsies were performed repeatedly from the ischemic and the non-ischemic zones of the left ventricle at an interval of 1–10 min until 1.5 min after the onset of VF or until 30 min after ligation if no VF occurred. Biopsy samples were frozen immediately in liquid nitrogen. The frozed myocardium was homogenized in a 2.5 ml of ice-cold 0.1N perchloric acid and centrifuged at 1600 x g for 20 min at 4°C.

**Determination of Norepinephrine**

Catecholamines were purified and concentrated by an extraction with alumina according to the method of Anton and Sayer.6 The following procedures were carried out: 50 mg alumina and 2 ml of 2M Tris HCl with EDTA and DTT were added to each tube (pH 8.55–8.60). After shaking the tubes thoroughly for 10 min, liquid was removed and the alumina was washed 3 times with water. After the last washing the

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supernatant was removed as completely as possible and the catecholamines were desorbed with a 200 µl of 0.1N HCl. Then, 25 µl of supernatant was injected into the column and analyzed by high-performance liquid chromatography with electrochemical detection (Yanagimoto Model L-2000, column: Yanapak ODS, detector cell: VMD-101).

**Measurement of Cyclic AMP**

C-AMP was measured by the modified radio-immunoassay method.

Student's t-test, either the paired or unpaired test as appropriate, was used to compare mean values between the groups. A p value of less than 0.05 was considered significant. Values were expressed as mean ± SEM.

**RESULTS**

**Incidence of Ventricular Fibrillation in Control Dogs**

In the ligation-only group, VF was induced in 2 of 10 dogs (20%) after the LAD ligation (Table I). In the ligation plus punching biopsy group, VF was induced in 52 of 104 dogs (50%) after serial biopsies.

**Myocardial Norepinephrine and Cyclic AMP Concentration in Normal Dogs**

As shown in the upper part of Fig.1, the myocardial norepinephrine concentration was...
0.288 ± 0.034 mg/g wet tissue before the ligation of the LAD, and there were no significant differences between norepinephrine levels in the ischemic and the non-ischemic zones 5, 10, 20 and 30 min after the LAD ligation in dogs without VF. As shown in the lower part of the same figure, the myocardial c-AMP concentration was 0.722 ± 0.036 nmol/g wet weight before the LAD ligation. There was also no significant difference in the myocardial c-AMP levels between the ischemic and the non-ischemic zones 5, 10, 20 and 30 min after the ligation in dogs without VF.

Figure 2 shows the serial changes of the myocardial norepinephrine concentration in dogs with VF. It was 0.274 ± 0.042 μg/g tissue before the LAD ligation and significantly increased in both the non-ischemic and the ischemic zones to 0.400 ± 0.062 (p < 0.05) and 0.433 ± 0.065 μg/g (p < 0.001), respectively, one minute before the development of VF, and it decreased 1 and 2 min after its development.

Figure 3 shows that the serial changes of myocardial c-AMP concentration in dogs with VF were 0.739 ± 0.033 nmol/g tissue before the LAD ligation and significantly increased to 1.077 ± 0.087 in the ischemic zone (p < 0.05) 5 min before VF. This increase of c-AMP in the ischemic zone continued for 2 min after the development of VF. There were significant differences of c-AMP concentration between the ischemic and the non-ischemic zones 1 and 5 min before the development of VF (p < 0.005 and p < 0.05, respectively), but it reached the same level 2 min after the development of VF.

Incidence of Ventricular Fibrillation in Dogs Premedicated with Dibutyril Cyclic AMP

In 13 dogs premedicated with DBC-AMP before the LAD ligation, 7 (53.8%) developed VF after the ligation (Table I). This rate was significantly higher than that in the ligation-only group, but not significantly different from that in the ligation plus punching biopsy group.

Myocardial Norepinephrine and Cyclic AMP Concentrations in Dogs Premedicated with Dibutyril Cyclic AMP

Figure 4 shows serial changes of myocardial c-AMP concentrations in both the ischemic and the non-ischemic zones before and after VF occurrence in dogs premedicated with DBC-AMP. The myocardial c-AMP concentrations in these dogs were markedly higher than those in the control dogs. However, there was no significant difference in the c-AMP concentration between the ischemic and the non-ischemic zones before the development of VF. Thirty seconds after the onset of VF there occurred a significant difference of myocardial c-AMP concentration between the ischemic and the non-ischemic zones of these dogs (p < 0.05).
Incidence of Ventricular Fibrillation in Propranolol Premedicated Dogs

As shown in Table I, in 26 dogs premedicated with propranolol before the LAD ligation, 6 developed VF after the ligation. The incidence of VF in this group was 23.1%, being a significantly (p < 0.01) lower incidence as compared with that in the ligation plus puncturing biopsy group. By a $\chi^2$ test propranolol was found to effectively prevent VF by 26.9% in this experimental study.

Myocardial Norepinephrine and Cyclic AMP Concentrations in Dogs Premedicated with Propranolol

As shown in the upper part of Fig. 5, the myocardial norepinephrine concentrations of dogs premedicated with propranolol was 0.761 ± 0.092 ng/g wet tissue before the LAD ligation. After the ligation they maintained a significantly higher level in the ischemic zone as compared with the control dogs (p < 0.05 or p < 0.001). This consistently increased concentration of norepinephrine in the ischemic zone in dogs uncomplicated by VF may be due to a myocardial blockade of $\beta$-receptors by propranolol. However, as shown in the lower part of Fig. 5 myocardial c-AMP concentrations in the ischemic zone of dogs premedicated with propranolol were 0.656 ± 0.031 and 0.676 ± 0.033 nmol/g wet tissue 5 and 10 min after the ligation, respectively. These were significantly lower than those in dogs without propranolol (p < 0.05), and such a lower level continued throughout the experiment. As shown in Fig. 6, the myocardial c-AMP concentrations tended to increase in 6 dogs with propranolol before the development of VF, though they were significantly lower than those in dogs without propranolol premedication.

DISCUSSION

The present findings demonstrated a close relationship between the increased myocardial c-AMP concentration in the ischemic zone and the increased occurrence of VF. It was also shown that the incidence of VF was inhibited in dogs premedicated with propranolol. Wollenberger et al.\textsuperscript{11} have reported that myocardial c-AMP began to increase 5 sec after coronary occlusion and reached its maximum after 10–15 sec, and then the elevated level was maintained for at least 10 min. On the other hand, Podzuweit et al.\textsuperscript{12} have reported that in the ischemic zone of the baboon heart, an increase in the c-AMP level started about 10 min before the onset of VF. On the other hand, dogs with a ligation of the peripheral part of the LAD showed no such increase in myocardial c-AMP within one hour and VF failed to occur.

In our study, the concentration of norepinephrine and c-AMP in both the ischemic and the non-ischemic zones did not show any significant changes in dogs without VF. However, the concentrations of norepinephrine and c-AMP in the ischemic zone were elevated significantly one min before the development of VF as compared to those in the non-ischemic zone, and their high level continued for 2 min after the beginning of VF. The c-AMP concentration in the ischemic zone was significantly higher than that in the non-ischemic zone one and 5 min before the development of VF. They may be the predisposing factors for the occurrence of VF.

Podzuweit et al.\textsuperscript{5} have proposed that the development of VF in ischemic heart disease and myocardial infarction may be related to an accumulation of c-AMP in the ischemic zone. An increased c-AMP concentration in the myocardial cell may accelerate an inflow of Ca\textsuperscript{2+} and relates to an acceleration of glycogenolysis during ischemia. It also accelerates lipolysis both in adipose tissue and in the heart. The metabolic gradient between the ischemic and the non-ischemic zones in the myocardium, thus produced, may predispose the heart to VF. The same authors have also reported that dibutyryl c-AMP enhanced ventricular vulnerability to fibrillation by lowering the threshold for VF and by increasing the duration of the vulnerable period\textsuperscript{13}.

In our study, the incidence of VF in dogs premedicated with DBC-AMP increased significantly as compared with the ligation-only group. However, when compared with the ligation plus puncturing biopsy group, there was no significant difference in the incidence of VF. There was also a difference between the norepinephrine and the c-AMP concentrations. The increased norepinephrine concentration in both the ischemic and the non-ischemic zones may be explained by an augmented sympathetic nervous drive in the non-ischemic zone as well as in the ischemic zone. However, the concentration of c-AMP was elevated significantly only in the ischemic zone.

Mori\textsuperscript{14} in our laboratory has reported that adenyl cyclase activity in the ischemic zone increased significantly as compared with that in the non-ischemic zone 15 min after the LAD liga-

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tion. This tendency continued for 60 min, and phosphodiesterase activity in the ischemic zone did not change significantly. Recently Mukherjee et al. have reported that the occlusion of the proximal left anterior descending coronary artery for one hour increased significantly the number of beta adrenoreceptors in the ischemic left ventricular tissue without changing the number of muscarinic cholinergic receptors. Their results may explain the different c-AMP concentrations in the ischemic and the non-ischemic zones in our present study.

The myocardial norepinephrine concentration increased in the propranolol-pretreated group throughout the experiment as compared with the controls. This may be due to the competition of norepinephrine and propranolol for β-receptors, resulting in an increased concentration of norepinephrine in the sympathetic nerve ending. However, the myocardial c-AMP concentration decreased throughout the experiment and was inhibited significantly 5 and 10 min after the LAD ligation. It is postulated that this decrease of c-AMP reduced the occurrence of VF, which, on the other hand, was increased by elevated c-AMP concentration in the ischemic zone. Opie has reported that propranolol reduces the uptake of fatty acid and increases the uptake of glucose in both the ischemic and the non-ischemic zones, thus reducing the disparity of metabolism in the ischemic and the non-ischemic zones and producing beneficial effects in the early stage of myocardial infarction. As a clinical implication, recent preliminary data from the National Heart, Lung, and Blood Institute indicates that propranolol reduced postinfarction mortality by 26%.

The authors studied the causal relation between myocardial norepinephrine or c-AMP and the occurrence of VF immediately following acute myocardial ischemia in order to clarify the mechanism of the VF occurrence which may be responsible for sudden death in ischemic heart disease. The present findings suggest a close relationship between the increased myocardial c-AMP concentration in the ischemic zone and the increased occurrence of VF. The incidence of VF was inhibited by 26.9% in dogs premedicated with propranolol.

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