STUDIES ON ADENYL CYCLASE SYSTEM IN MYOCARDIUM (PART II) ADENYL CYCLASE SYSTEM IN MYOCARDIAL INFRACTION OF DOGS

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Many reports agree on the increase of CA concentration in blood and urine of patients just after acute myocardial infarction,[1) - 6)] especially in cases of complications such as arrhythmia, shock and congestive heart failure. Physiological actions of CA on the heart such as inotropic and chronotropic action are mediated by cyclic AMP. It is believed that the level of myocardial cyclic AMP is a result of a balance between its rate of synthesis, catalyzed by adenyl cyclase, and that of hydrolysis, catalyzed by phosphodiesterase.

In the present paper, myocardial CA, adenyl cyclase and phosphodiesterase activity in the infarcted area and the non-infarcted area were measured after the ligation of left anterior descending coronary artery of mongrel dogs to search for the role of CA and adenyl cyclase-cyclic AMP system in acute myocardial infarction.

MATERIALS AND METHODS

1. Production of myocardial infarction

Fourty four mongrel dogs, weighing 9 – 15 kg, were used. The thorax of mongrel dogs were opened under anesthesia induced by intravenous injection of 30 mg/kg of sodium hexobarbital. Under artificial respiration, myocardial infarction was produced by the ligation of the proximal part of the left anterior descending coronary artery. Myocardial ischemia was ascertained by ST segment elevation of ECG monitoring by direct epicardial leads. (Fig. 1) Free walls of the left ventricle were removed directly from the open thorax and used for each assay. The dogs were divided into six groups: Nine dogs of 15 minutes after ligation of the left anterior descending coronary artery are abbreviated by $MI_{15'}$, six of 60 minutes by $MI_{60'}$, six of 2 days to MI_2, seven of 7 days by MI_7 and six of 30 days by MI_{30}.

2. Preparation of samples and determination of myocardial CA, adenyl cyclase activity and phosphodiesterase activity were described in part I. The result were analyzed using student's "t" test.

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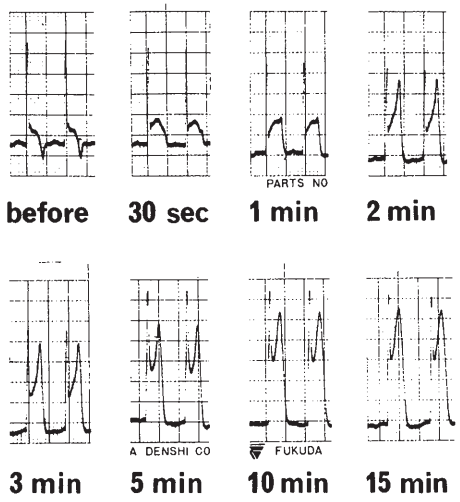

Fig. 1. One case of ECG monitoring.

RESULTS

1. CA concentration in the left ventricle

Mean concentration of CA of the left ventricle of 10 normal dogs was 1.00 ±0.081 μg/g (±S.E.M.). In the infarcted area it was 0.77 ±0.077 μg/g in $MI_{15'}$ and 0.80 ±0.077 μg/g in $MI_{60'}$, and both showed a slight reduction when compared with normal. The CA concentration of the infarcted area of MI_2, MI_7 and MI_{30} groups showed significant reduction. In the marginal area and non-infarcted area, the ventricular CA concentration showed slight decrease at 15 minutes and 60 minutes after the ligation and the most pronounced reduction was observed 2 days after, which averaged 0.39 ±0.056 μg/g in marginal area and 0.51 ±0.081 μg/g in non-infarcted area of MI_2 respectively. This reduction recovered fairly after 7 days and almost to normal 30 days after. (Fig. 2)

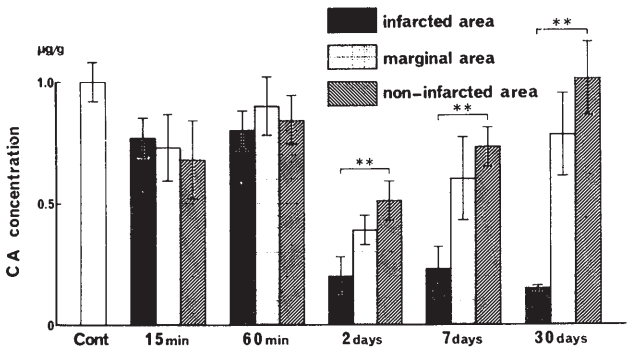

Fig. 2. CA concentration in the left ventricle of dogs in six groups.
**: $p < 0.01$

2. Adenyl cyclase activity in the left ventricle

The adenyl cyclase activity in the left ventricle of 6 normal dogs averaged 0.975 ±0.13mμ mole/h/mg (±S.E.M.). The activity of infarcted area in $MI_{15'}$ was higher than that of normal

dogs, and averaged 1.17 ±0.15mμ mole/h/mg, which was significantly elevated compared with non-infarcted area in $MI_{15'}$, (0.77 ±0.08mμ mole/h/mg) ($p < 0.05$). The adenyl cyclase activity of $MI_{60'}$, MI_7 and MI_{30} groups were lower than that of normal dogs. In $MI_{60'}$, it averaged 0.57 ±0.084mμ mole/h/mg, which was still higher than that of non-infarcted area, (0.21 ±0.05mμ mole/h/mg). But after 2 days and 7 days, the adenyl cyclase activity of the infarcted area which had been lower than that of non-infarcted area, gradually decreased, and averaged 0.29 ±0.05mμ mole/h/mg in MI_2, 0.22 ±0.08mμ mole/h/mg in MI_7 and 0.15 ±0.04mμ mole/h/mg respectively. (Fig. 3)

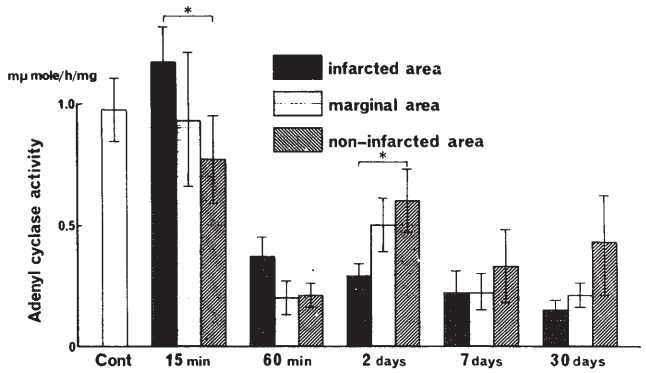

Fig. 3. Adenyl cyclase activity in the left ventricle of dogs in six groups.
*: $p < 0.05$

3. Phosphodiesterase activity in the left ventricle

The phosphodiesterase activity in the ventricle of 6 normal dogs averaged 140 ±7.45mμ mole/h/mg (±S.E.M.). There were no significant differences in activity between infarcted, marginal and non-infarcted areas, each of which averaged about 135mμ mole/h/mg. The activity showed a little reduction in $MI_{60'}$. But on 2 days and thereafter, the activity of infarcted area was gradually reduced, and averaged 72.23 ±7.11mμ mole/h/mg in MI_2, 51.8 ±9.24mμ mole/h/mg in MI_7 and 34.35 ±3.23mμ mole/h/mg in MI_{30} respectively. In non-infarcted area, the activity was reduced a little compared with normal and remained almost constant. (Fig. 4)

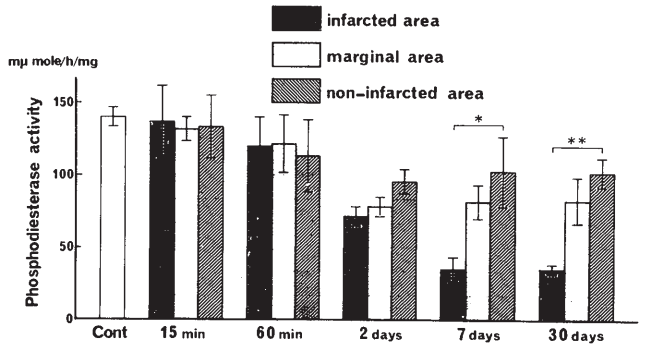

Fig. 4. Phosphodiesterase activity in the left ventricle of dogs in six groups.
*: $p < 0.05$ **: $p < 0.01$

DISCUSSION

The present experiment showed a slight decrease of CA concentration in the infarcted and non-infarcted areas of the left ventricle after 15 and 60 minutes of ligation compared with normal without statistical significance. Many reports showed the depletion of myocardial CA in acute myocardial infarction,[7) – 8)] and the present study showed the same tendency. The mechanism of myocardial CA depletion in acute myocardial ischemia is considered chiefly to be due to accelerated excretion of CA from the myocardium. On the other hand, blood CA is known to increase in myocardial infarction.[1), 4)] It is considered that the elevated blood CA is not only caused by the excretion of myocardial CA into the circulation due to the ischemia, but also by the sympathetic nervous system and adrenals due to stress such as pain or of the lung edema.[2)] Moreover, a remarkable diminution of CA in the infarcted area on 2, 7 and 30 days after ligation was due to the development of myocardial necrosis. As already described, inotropic and chronotropic responses of CA to myocardium is considered to be mediated by myocardial cyclic AMP. So, the investigation of myocardial adenyl cyclase-cyclic AMP system in myocardial ischemia seemed to be very important to understand the pathobiochemical changes of acute myocardial ischemia. At present, there are a few studies in this field. In the present experiment, adenyl cyclase activity in the infarcted area of the left ventricle was increased compared to that of the non-infarcted area after 15 minutes of the ligation. Sixty minutes after the ligation, all myocardial adenyl cyclase activity showed depressions due to development of myocardial necrosis.

But adenyl cyclase activity in the infarcted area continued to be relatively higher than that of the non-infarcted area at 60 minutes after the ligation. Phosphodiesterase activity in the ventricle showed no significant change in infarcted and non-infarcted areas at 15 and 60 minutes after the ligation. Phosphodiesterase activity on 2, 7, and 30 days after ligation showed significant depression such as adenyl cyclase activity.

Wollenberger[9)] reported that myocardial cyclic AMP began to be elevated 5 seconds after coronary occlusion and reached maximum values after 10 to 15 seconds. The elevated level was then maintained for at least 10 minutes. He pointed out that the rapid initial rise in cardiac cyclic AMP within 1 minute following the arrest of blood flow was due to increase of adenyl cyclase activity by excreted noradrenaline and that the prolonged maintenance of the elevated cyclic AMP level in the ischemic tissue may conceivably, and at least in part, have the consequence of an accumulation of acid products of glycolysis, causing a decrease in the activity of cyclic nucleotide phosphodiesterase. But he did not measure the cardiac phosphodiesterase activity. In this study, the cardiac phosphodiesterase activity was measured and showed no significant changes, and only adenyl cyclase activity was elevated significantly. The mechanism of elevated cardiac adenyl cyclase activity in the early stage of myocardial ischemia is not fully clear, but it is not a secondary phenomenon due to the changes of myocardial CA, because CA in infarcted area is not decreased. The change of adenyl cyclase activity could be due to the removal of certain inhibitors of this enzyme from the cell membrane as McNamara[10)] had pointed out. Of course, the possibility of blood CA elevation in early stage of myocardial ischemia is not neglectable.

The transient elevation of myocardial adenyl cyclase activity in the early stage of acute myocardial infarction is not fully concluded from present results. In addition to the rise of blood CA in the early stage of myocardial infarction, myocardial adenyl cyclase activity was elevated and unequal adenyl cyclase activity in parts of infarcted and non-infarcted areas

was noticed. This indicates that the cyclic AMP production would be unequal in the early stage of myocardial infarction. At present, the mechanism of arrhythmias with acute myocardial infarction has been chiefly studied electrophisiologically, and not from biochemical standpoints such as myocardial CA and cyclic AMP. The elevation of myocardial adenyl cyclase activity and the unequal distribution of cyclic AMP production were notable facts in relation to the occurrence of arrhythmias at the early stage of myocardial infarction. Adenyl cyclase activity in the infarcted area was gradually decreased 2, 7, and 30 days after ligation of the coronary artery.

In the non-infarcted area, adenyl cyclase activity showed recovery in 60 minutes and after, but still was lower compared with normal. The reason for these drops of adenyl cyclase activity in non-infarcted area is considered to be due to hyperfunction to compensate for the dysfunction of infarcted area. Adenyl cyclase activity in non-infarcted area is considered primarily depressed in the same manner in cardiac hypertrophy and impending heart failure described in part I, and the decrease of its activity may play a role in the mechanism of cardiac failure after myocardial infarction.

SUMMARY

In order to study the pathophysiological role of CA and adenyl cyclase-cyclic AMP system in the early stage of myocardial infarction, CA, adenyl cyclase activity and phosphodiesterase activity in the infarcted and non-infarcted areas in experimental infarction of dogs were measured.

1. CA concentration in the infarcted area showed little change compared to non-infarcted area 15 minutes and 60 minutes after coronary artery ligation, but significant was reduced after 2 days and thereafter.
2. Adenyl cyclase activity in the infarcted area was significantly increased compared to the non-infarcted area at 15 minutes, and this tendency had continued while 60 minutes after, but decreased after 2 days and thereafter.
3. Phosphodiesterase activity in the infarcted area was not significantly changed in the early stage of myocardial infarction, and there had been gradual diminution after 2 days and later.

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