Myocardial and Plasma Levels of Adenosine 3':5'-Cyclic Phosphate*

Studies in Experimental Myocardial Ischemia

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Alterations in myocardial and plasma levels of adenosine 3':5'-cyclic phosphate (cyclic AMP) were studied following clamping of the aorta or coronary artery occlusion in 30 dogs. Plasma cyclic AMP levels increased markedly after thoracotomy but returned to control levels two hours later. Complete arrest of aortic flow (clamping) induced a significant early increase in the myocardial cyclic AMP levels of all animals studied. No increase was noted following pretreatment with propranolol or sham-occlusion. After localized coronary occlusion, only modest and insignificant changes occurred in plasma cyclic AMP levels in anesthetized animals and also in conscious dogs. The present study suggests that adrenergically mediated changes in tissue cyclic AMP content are an early manifestation of both generalized and local myocardial ischemia, while the plasma cyclic AMP level is a relatively insensitive indicator of small coronary occlusions.

Several experimental and clinical studies have been performed to define the biochemical and metabolic changes associated with myocardial anoxia or ischemia, or both. One of the early changes produced is increased glycogenolysis with reduction of the glycogen level in myocardial cells after five minutes of ischemia. This effect is due to activation of phosphorylase-b kinase and glycogen phosphorylase and is associated with release of endogenous cardiac norepinephrine. Since these effects were suppressed by β-blockers, it is presumed that the catecholamine-mediated effects on adenylyl cyclase activity produce increased levels of adenosine 3':5'-cyclic phosphate (cyclic AMP), which in turn activate phosphorylase-b kinase, in the acutely ischemic myocardium.

Surprisingly few studies have been conducted to substantiate the details of this hypothesis. Wollenberger et al showed that circulatory arrest in dogs, produced by transection of the aorta, induced an immediate rise in cardiac cyclic AMP levels. On the other hand, Robison et al found that the cyclic AMP content of the rat heart did not change after three minutes of perfusion with an oxygen-free solution.

Recently we characterized plasma levels of cyclic AMP in patients with acute myocardial infarction and noted a good correlation between plasma levels, hemodynamics, and prognosis. Studies of arterial and coronary sinus levels suggested that at least some of the cyclic AMP was released from the heart. Accordingly, the present study was performed in the dog to evaluate changes in myocardial and plasma cyclic AMP levels following ischemia and myocardial infarction.

**METHODS**

*Open-Chest Anesthetized Dogs*

Normal mongrel dogs weighing 20 to 25 kg (44 to 55 lb), under thiopental (Pentothal) sodium anesthesia and positive-pressure breathing, had catheters inserted into the femoral artery and vein and underwent electrocardiographic and arterial pressure monitoring. The thorax and pericardium were then opened. Since plasma cyclic AMP levels were increased by thoracotomy (Table 1), an interval of two hours was allowed between the completion of thoracotomy and subsequent studies.
Table 1—Changes in Venous Plasma Cyclic AMP Levels Induced by Thoracotomy

<table>
<thead>
<tr>
<th>Cyclic AMP (pmol/ml)</th>
<th>Control Samples (Conscious Dogs)</th>
<th>After Anesthesia and Catheter Insertion</th>
<th>After Thoracotomy*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog</td>
<td>5 min</td>
<td>30 min</td>
<td>1 hr</td>
</tr>
<tr>
<td>1</td>
<td>6.5</td>
<td>11.0</td>
<td>15.5</td>
</tr>
<tr>
<td>2</td>
<td>7.5</td>
<td>...</td>
<td>10.0</td>
</tr>
<tr>
<td>3</td>
<td>3.1 → 4.1</td>
<td>...</td>
<td>9.5</td>
</tr>
<tr>
<td>4</td>
<td>5.5 → 6.0</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>5</td>
<td>14.0 → 13.0</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>6</td>
<td>28.0</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>45.0</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

*Including pericardectomy and preparation of the coronary vessels for ligation.

**Group A.** Clamping of the aorta was studied in ten dogs. A control biopsy of 50 to 100 mg of tissue was taken from the left ventricular anterior wall, after which the aorta was cross-clamped 10 to 15 mm from its origin with a resulting fall of arterial pressure to zero (Fig 1). Direct left ventricular puncture for pressure recording showed an initial rise in pressure and subsequent gradual decrease. Sequential biopsies from the area adjacent to the first biopsy were obtained, beginning five seconds after clamping. In a subgroup of three animals, propranolol (0.5 mg/kg of body weight) was administered intravenously 20 minutes before clamping of the aorta.

**Group B.** Partial clamping of the aorta was studied in six dogs. These animals underwent the same procedures as those from group A, with the exception that the aorta was only partially clamped with no subsequent fall in the arterial pressure. Sequential biopsies were taken in the same manner.

**Group C.** Occlusion of the left anterior descending coronary artery (LAD) was studied in nine dogs. Control blood samples were taken two hours after thoracotomy. In some of the animals, blood samples were also taken before and after thoracotomy. Following occlusion of visible collaterals, the LAD was occluded. Blood samples were taken at five minutes postocclusion and at 30-minute intervals up to four hours. Myocardial ischemia was documented by electrocardiographic changes, localized cyanosis, and systolic bulging.

**Closed-Chest Conscious Dogs**

**Group D.** This group of five dogs was submitted to a two-step procedure consisting of the following: (1) Thoracotomy was performed under sterile conditions, and a string or an inflatable cuff was placed around the middistal LAD or around one of its major branches. The string was passed through a polyethylene tube, which was exteriorized through an orifice in the chest wall. A venous line was left in place. (2) One week after thoracotomy, the conscious dog was monitored electrocardiographically, and several control blood samples were taken. Then the string was tied or the cuff inflated. Myocardial ischemia was confirmed by an injury pattern on the electrocardiogram. Sequential blood samples were taken at various intervals, and the animals were subsequently killed.

**Tissue Samples**

The tissue samples (50 to 100 mg) for cyclic AMP determinations were surgically removed from an area free of visible vessels, immediately introduced into tubes containing 3 ml of a 1:1 mixture of a 12 percent perchloric acid solution and a 95 percent ethyl alcohol solution at −10°C and homo-

**EFFECT OF AORTIC CLAMPING ON ARTERIAL PRESSURE**

![Figure 1. Electrocardiogram and aortic pressure before and after complete clamping of aorta in representative dog (group A).](http://journal.publications.chestnet.org/pdfaccess.ashx?url=data/journals/chest/20968/ on 05/31/2017)
genized with a tissue homogenizer (Tekmar Tissuemizer) for 15 to 30 seconds. The elapsed time from the biopsy to the homogenization was five to ten seconds.

**Blood Samples**

Blood (3 to 5 ml) was collected in heparinized chilled tubes. The plasma was immediately separated by centrifugation, and a barium-zinc precipitation was performed, as previously described.24

**Biochemical Methods**

Plasma and tissue cyclic AMP levels were measured using a previously described method which is a saturation assay based on competitive binding with a protein from adrenal glands.24

After homogenization, the tissue samples were centrifuged for ten minutes at 15,000 rpm and 5°C. The supernatant was removed and neutralized with solid potassium bicarbonate. The neutralized supernatants were centrifuged at 15,000 rpm for ten minutes. One milliliter of the supernatant was twice precipitated with barium and zinc solutions (0.1 M barium hydroxide and 0.1 M zinc sulfate), with a resultant neutral clear supernatant.

The preparation was stored frozen at −5°C until assay. A reagent blank was also prepared. This consisted of a barium-zinc precipitated, neutralized perchloric acid-alcohol mixture, with no tissue aliquot.

The tissue cyclic AMP content was measured by the same saturation assay described for plasma, with the following two modifications: (1) The standard curve contained an aliquot of tissue blank equal to that used for the assay. (2) Two picomols of unlabeled cyclic AMP were added to each tissue sample in triplicate for the purpose of checking recovery. This modification was required because of the presence of inhibitory or acceleratory substances, or both, within the tissue aliquot.25

Protein determination by the method of Lowry et al.26 was done on the precipitated portion of the homogenized tissue after digestion with sodium hydroxide.

**RESULTS**

The hemodynamic effects of complete clamping of the aorta (group A) are illustrated in Figure 1. Distal arterial pressure rapidly declined (four seconds) and stabilized at a low level. The effects of complete clamping of the aorta on cyclic AMP levels of the left ventricular myocardium are illustrated in Figure 2. The control levels of the myocardial cyclic AMP varied between 6.5 and 31.4 pmol/mg of protein. In each one of the seven dogs illustrated in the upper panel of Figure 2, an increase in myocardial cyclic AMP content was found during the first two minutes after circulatory arrest; however, the magnitude of the changes and the time to peak effect were

![EFFECT OF COMPLETE CLAMPING OF THE AORTA ON THE MYOCARDIAL CYCLIC AMP LEVELS OF DOGS](image-url)

Figure 2. Myocardial cyclic AMP levels in sequential biopsies taken from dogs before and after complete clamping of aorta (group A). Upper panel (seven dogs) illustrates alterations in tissue cyclic AMP content induced by clamping. Lower panel (three dogs) illustrates blockade of these effects by propranolol pretreatment (0.5 mg/kg of body weight).
quite variable.

The lower panel of Figure 2 illustrates the effect of propranolol pretreatment (0.5 mg/kg of body weight) on the cyclic AMP levels of the left ventricular myocardium after total clamping of the aorta. The control cyclic AMP levels were decreased after propranolol administration. In contrast to the untreated animals, animals pretreated with propranolol failed to show any increase in tissue cyclic AMP levels after clamping of the aorta.

Figure 3 illustrates the results obtained in group B (six dogs), which had partial or pseudo clamping of the aorta without any change in arterial pressure. The cyclic AMP levels obtained from sequential biopsies did not show an increase. In particular, the results obtained in this group (Fig 3) are quite similar to those seen in the dogs pretreated with propranolol (Fig 2, lower panel).

Group C (nine dogs) consisted of the open-chest anesthetized dogs with LAD occlusion. Five dogs had a distal LAD occlusion (“small-size” infarction), and four had a mid LAD occlusion (“medium-size” infarction).

Figure 4 illustrates the venous plasma cyclic AMP levels from five minutes to four hours postocclusion. Only one of the animals died spontaneously (two hours postocclusion). Thirty minutes before death, this dog became hypotensive and died in ventricular fibrillation. This was the only animal that exhibited a marked increase in the venous plasma cyclic AMP levels, despite the rather high initial level. In the other animals with moderate or small infarctions, there were fluctuations in the cyclic AMP levels with a slight tendency to increased levels in some animals. Several dogs, however, did not show any significant change.

The five animals of group D were conscious closed-chest dogs. They all had “small-size” infarctions.

**MYOCARDIAL CYCLIC AMP LEVELS IN SHAM-OPERATED DOGS**

![Figure 3](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/20968/)

**Figure 3.** Sequential myocardial cyclic AMP levels in biopsies taken from six animals (group B) with partial or pseudo clamping of aorta.

**PLASMA CYCLIC AMP LEVELS IN OPEN-CHEST DOGS WITH CORONARY OCCLUSION**

![Figure 4](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/20968/)

**Figure 4.** Venous plasma cyclic AMP levels in nine anesthetized open-chest dogs (group C) before and after mid (medium-size infarction) or distal (small-size infarction) LAD occlusion.

**DISCUSSION**

The present study was designed to investigate changes in tissue cyclic AMP level with general ischemia and changes in venous cyclic AMP level with regional localized myocardial ischemia.

**PLASMA CYCLIC AMP LEVELS IN CONSCIOUS DOGS WITH DISTAL CORONARY OCCLUSION**

![Figure 5](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/20968/)

**Figure 5.** Plasma cyclic AMP levels in five conscious dogs before and after distal LAD occlusion.
With total occlusion of aortic flow, there was a significant increase in the cyclic AMP levels of the left ventricular myocardium within the first one to two minutes after occlusion (Fig 2). This increase was not sustained for longer periods, even in the presence of continued ischemia and subsequent cardiac arrest.

Since animals pretreated with propranolol did not show an increase in tissue cyclic AMP level, this effect was presumably due to local release of catecholamines. These data are thus similar to that of Wollenberger et al, who also noted an early increase in myocardial cyclic AMP level following ischemia, which could be blocked by propranolol administration. The fact that their effects were more sustained than those of the present study may reflect differences in the experimental preparation (aortic transsection with exanguination vs aortic clamping). Certainly the present study was associated with stagnation of poorly oxygenated blood and the delayed clearance of metabolic end products, which were absent in the study of Wollenberger et al. In any event the mechanism in both studies was probably related to a widespread sympathetic discharge in response to the abrupt fall in blood pressure.

In the second part of the current study, we induced coronary occlusion in open-chest anesthetized and conscious dogs and then measured alterations of plasma cyclic AMP level after the occlusion. In a parallel clinical study, there was a tendency to increased levels of plasma cyclic AMP in patients with acute myocardial infarction during the first day of illness. Furthermore, there were elevated levels in patients who died within a week of the infarction.

In the present study the only open-chest animal who died during the experiment (Fig 4) was hypotensive and exhibited markedly increased levels of plasma cyclic AMP. In the group of conscious dogs, only small infarctions were produced, and there was a minor tendency towards increased levels of cyclic AMP after occlusion (Fig 5) as compared to control animals. The sequence of events appears to be as follows: ischemic “stimuli”→generalized sympathetic discharge with release of catecholamines within the tissue→activation of adenyl cyclase system→increased content of tissue cyclic AMP→escape of the cyclic AMP into the extracellular spaces→increased plasma cyclic AMP concentration. It is apparent, however, that changes in plasma cyclic AMP level were minimal and might be masked by spontaneous fluctuations.

Elevations of cyclic AMP level represent compensatory attempts to maintain the integrity of the cardiovascular system. Unanswered questions, however, relate to the potential role of cyclic AMP in the genesis of arrhythmias, the viability of ischemic myocardium, and its effect on other subcellular processes during ischemia. Further studies should help to clarify the role of cyclic AMP in this regard and to define its role in the setting of acute myocardial infarction.

REFERENCES
18. Wollenberger A, Krause EG: Activation of a glucan phosphorylase and related metabolic changes in dog myocardium following arrest of blood flow. Biochim Biophys...
ANNOUNCEMENTS

International Tuberculosis Conference

The 23rd International Conference, organized by the International Union Against Tuberculosis, will be held in Mexico City, Mexico, September 22-26. For information, please contact the Union at 3 Rue Georges Ville, 75116 Paris, France.

International Symposium on Pediatric Otorhinolaryngology

The Children's Mercy Hospital, in cooperation with the University of Missouri-Kansas City School of Medicine and Southwest Pediatric Society will present an International Symposium on Pediatric Otorhinolaryngology, September 11-13. The faculty will include teachers from Europe, as well as the U.S., Canada and Mexico. For information, please write Dr. B. Jazbi, Children's Mercy Hospital, Kansas City, Missouri 64108.

28th Annual Symposium on Pulmonary Disease

Fitzsimons Army Medical Center, Denver, will be the site for the 28th Annual Symposium on Pulmonary Disease, September 8-12. The symposium is jointly sponsored by the Pulmonary Disease Service and the Allergy-Immunology Service at Fitzsimons Hospital. For further information and application, contact Roald A. Nelson, M.D., Program Director, Fitzsimons Army Medical Center, Denver 80240.

Pulmonary Radiology and the Altered Immune States

The University of Wisconsin will present the course, Pulmonary Radiology and the Altered Immune States, August 17-20 at Telemark Lodge, Cable, Wisconsin. For information, contact Francis F. Buzicka, M.D., c/o Continuing Medical Education, 610 Walnut Street, Madison, Wisconsin 53706.

CDC Courses

The Center for Disease Control, USPHS, Atlanta, will present a series of courses in special subdivisions of microbiology during the period July 14, 1975-June 25, 1976. These courses will emphasize laboratory diagnostic procedures and public health problems in bacteriology, mycology, parasitology and virology. Information and applicants forms may be obtained from the Bureau of Laboratories, CDC, Atlanta 30333.