Stabilization of a Derangement in Adenosine Triphosphate Metabolism During Sustained, Partial Ischemia in the Dog Heart

WILLIAM A. NEILL, MD, FACC,* JOANNE S. INGWALL, PhD,† with the technical assistance of ELOISE ANDREWS, MALAVALLI A. GOPAL, KAREN KLEIN, MARTHA KRAMER, JOHN M. OXENDINE, Z. HARRY PIOTROWSKI, ILANA REIS

Boston, Massachusetts

Severe myocardial ischemia in dogs (perfusion 10% of normal) caused progressive deterioration in adenosine triphosphate (ATP) metabolism. Between ½ hour and 5 hours, myocardial ATP content fell from 55 to 6% of normal, and the sum of adenine nucleotides fell from 66 to 14% of normal. Moderate ischemia (perfusion 20 to 70%) also disturbed ATP metabolism, but to a lesser degree. Moreover, there was no significant change in the concentration of any ATP metabolite between 1½ hour and 5 hours of moderate ischemia. ATP content was 66 and 52% of normal, and adenine nucleotide content was 73 and 59% of normal at ½ hour and 5 hours, respectively. Trivial ischemia (perfusion 80% or greater) barely perturbed ATP metabolism at either ½ hour or 5 hours. Thus, in contrast to severe or trivial ischemia, prolonged moderate ischemia produced a derangement in ATP metabolism that persisted and was relatively stable for 5 hours.

(J Am Coll Cardiol 1986;8:894-900)

Normal cardiac metabolism and function depend on continual nutritional support from coronary perfusion. Occlusion of the coronary artery of a dog is followed by decay of myocardial contraction (1) and later by a progressive decline in myocardial adenosine triphosphate (ATP) concentration (2,3). The longer the period of occlusion, the slower the return toward normal function and metabolism. If reinstallation of coronary blood flow is delayed for as long as 12 to 15 minutes, full recovery to preoclusion contraction strength and ATP levels requires several days (4–7). Beyond 30 minutes, restoration of ATP and mechanical function is incomplete, and ultrastructural damage occurs (8–10).

The consequences of intense myocardial ischemia have been extensively investigated in experiments employing sudden complete occlusion of a coronary artery. There is comparatively little information about the effects of mild or moderate ischemia on mechanical function or ATP metabolism. One study (11) indicated that with less intense myocardial ischemia, contraction strength was less profoundly affected and recovered completely, even after 5 hours. The possibility that intermediate degrees of ischemic dysfunction can be tolerated and are potentially reversible after a prolonged period has important implications for treatment of patients with coronary heart disease who may have varying degrees of ischemia.

The experiments described here were designed to answer the following questions: 1) Do slight or moderate decreases in coronary blood flow affect myocardial ATP metabolism? 2) With sustained ischemia, does ATP metabolism, once disturbed, always progressively deteriorate or can an abnormal metabolic state stabilize? To address these questions we developed a model in dogs using subtotal coronary stenosis to produce regional myocardial ischemia, and we measured the myocardial concentrations of ATP and its primary catabolites after ½ hour or 5 hours of stable partial ischemia.

Methods

Experimental preparation. Twenty-eight mongrel dogs, pretreated with acetylsalicylic acid, 300 mg, to inhibit platelet aggregation, were anesthetized with halothane after premedication with acepromazine, 10 mg, and methohexital induction. They were endotracheally intubated and ventilated with 1½% halothane in oxygen for the duration of the experiment. The heart was exposed by a left thoracotomy.

From the *Boston Veterans Administration Medical Center, Boston, Massachusetts, and the †Department of Medicine, Brigham and Women’s Hospital and Harvard Medical School, Boston. This study was supported by Grant M19S 6004 from the Veterans Administration and Grant HC 26215 from the Specialized Center of Research, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland.

Manuscript received November 13, 1985; revised manuscript received April 2, 1986, accepted April 9, 1986.

Address for reprints: William A. Neill, MD, MacNeal Hospital, 3249 South Oak Park Avenue, Berwyn, Illinois 60402.

©1986 by the American College of Cardiology

0735-1097/86/$3.50
A segment of the proximal left circumflex artery was encircled by a Statham electromagnetic flowmeter transducer and 5 mm distally by a balloon cuff occluder. Heparin was administered, the thoracotomy was covered by towels and a heating pad was used to maintain body temperature. Physiologic saline solution was infused intravenously at approximately 80 ml/hr. Ventilation was controlled to keep arterial blood partial pressure of oxygen (P'O2) above 100 mm Hg and pH near 7.40. Thoracic aortic pressure was recorded from a catheter introduced through a carotid artery.

The balloon cuff occluder was connected by a tube to a mercury reservoir. To induce regional myocardial ischemia, the reservoir was gradually elevated until enough mercury entered the balloon to reduce the arterial flow to the desired value, as registered by the flowmeter transducer. This is an isobaric system that compensates automatically for unintended physical changes at the stenosis site, for example, ligature loosening or arterial wall edema. Compared with a closed system, however, it is sensitive to changes in opposing intraluminal coronary pressure. The mercury reservoir was lowered slightly to balance the decreases in aortic and coronary pressures that occurred during the first few minutes of ischemia, keeping monitored arterial flow constant. Subsequent readjustments were rarely needed to maintain blood flow constant during the ischemic period. Ventricular arrhythmias were minimal, and no dog died prematurely.

**Myocardial biopsy.** A single myocardial biopsy specimen for metabolic analysis was taken in each experiment after a period of controlled ischemia had been maintained for either ½ hour (10 experiments) or 5 hours (10 experiments). The biopsy procedure failed in two other dogs whose data are not presented. The beating heart was reexposed, taking care not to disturb its perfusion as monitored by the flowmeter tracing. The biopsy specimen was obtained from the central ischemic region of the left ventricle (posterior to the obtuse marginal branch of the left circumflex artery). One operator slid his finger under the apex and rotated the heart slightly counterclockwise to move the lateral surface anteriorly. Another operator immediately obtained the transmural biopsy tissue by a motor-driven hollow steel drill 1 cm in diameter (12). Within 3 seconds of the initial contact between drill and heart, the biopsy tissue was compressed by a Wollenburger clamp precooled in liquid nitrogen. The frozen myocardial wafer was broken into endocardial and epicardial halves for subsequent biochemical analyses.

The resulting pellet was dissolved in 1 N sodium hydroxide and analyzed for protein content using the method of Lowry et al. (13). Values are expressed as nanomoles per milligram cardiac protein.

**Control groups.** Control biochemical data were from eight myocardial samples taken from six dogs. In four dogs a transmural sample was obtained from the region of the left ventricular free wall supplied by the left anterior descending artery after 4½ hours of left circumflex stenosis, and in two dogs a transmural sample (subsequently divided into subendocardial and subepicardial portions) was obtained from the left circumflex region after 5 hours in a sham experiment without coronary stenosis.

**Regional myocardial blood flow.** Regional myocardial blood flow was determined by the microsphere technique, introducing approximately 1 million radioactive spheres (15 μ diameter) through a left atrial catheter during a 30 second period, while a reference arterial blood sample was withdrawn from the aorta (14). After the biopsy procedure, the dog was killed and the heart was removed. We cut the left ventricular free wall into regions supplied by the left anterior descending and left circumflex arteries, discarding the apex and a 1 cm wide border from each region. The remaining central nonischemic and ischemic regions were further subdivided into subendocardial and subepicardial halves. The mean amounts of myocardium analyzed for different regions were: left anterior descending subendocardium, 9.9 g and subepicardium, 11.4 g, left circumflex subendocardium, 10.3 g and subepicardium, 12.1 g.

Baseline regional coronary blood flow was determined by microsphere injection in each experiment. In the ½ hour experiments, regional coronary blood flow was repeated immediately before securing the biopsy. In the 5 hour experiments, separate blood flow determinations were made at ½, 2½ and 5 hours of left circumflex stenosis. Radioactive emissions of reference blood samples and myocardial samples were counted in a Packard 5230 automatic gamma spectrometer system.

**Statistics.** Statistical significance of differences in hemodynamic data was determined in the ½ hour experiments by a two-tailed paired t test. In the 5 hour experiments, possible trends in sequential measurements were assessed by analysis of variance, using the SAS GLM procedure (15). For metabolite concentrations, comparisons were made by using the Neuman-Keuls multiple range test, which tests the null hypothesis that the pairs being compared are equal. The comparisons were made with the aid of the Statistics and Data Management Package (Bolt, Beranek and Newman) and the VAX System (Digital Equipment Corporation).

Experiments were conducted in accordance with animal welfare regulations at the Boston Veterans Administration Medical Center and were in line with the principles of the American Physiological Society.

**Results**

At baseline study, the left circumflex artery blood flow tracing registered by the flowmeter transducer exhibited a normal phasic waveform, with relatively high diastolic flow and lower systolic flow. The mercury reservoir was raised
until left circumflex mean flow fell to a level between 17 and 70% of baseline, depending on the experiment. In every experiment, in the presence of the stenosis, the flow tracing became damped with systolic flow at least as high as diastolic flow, as is characteristically found in ischemia (16).

**Hemodynamic data (Fig. 1).** Left circumflex stenosis caused a decrease in aortic blood pressure (p < 0.01). Heart rate increased slightly in the ½ hour experiments (p < 0.05) but not in the 5 hour experiments. Regional myocardial flow data, determined by the microsphere method, are given separately for the subendocardial and subepicardial layers of the left ventricular territory supplied by the stenotic artery. Flow decreased in both layers (p < 0.001) and the degree of ischemia was proportionately greater in the subendocardium (subendo) (open circles) than in the subepicardium (subepi) (closed circles). There was no significant difference by the stenotic artery. The decrease in flow was proportionately greater in the subendocardium (subendo) (open circles) than in the subepicardium (subepi) (closed circles). There was no significant trend in nonischemic blood flow between ½ and 5 hours.

**Grades of myocardial ischemia.** The intensity of myocardial ischemia was judged by the ratio of blood flow between the ischemic and nonischemic regions. The mean baseline left circumflex to left anterior descending blood flow ratio was 0.97. To examine the influence of the intensity of ischemia on ATP metabolism, all myocardial samples from ½ and 5 hour experiments were collated into three grades according to their blood flow ratio during ischemia. In the 5 hour experiments, the mean ratio value for the three myocardial blood flow determinations was used. Because the intensity of ischemia differed between subendocardial and subepicardial layers, we treated these halves of each biopsy specimens as separate samples. In 13 samples, the blood flow ratio ranged from 0.8 to 1.1, which was defined as trivial ischemia. In 21 samples, the ratio ranged from 0.2 to 0.7, which was defined as moderate ischemia. In six samples, the ratio was 0.1; this value is comparable with complete coronary occlusion and was termed severe ischemia. In Figure 2, myocardial blood flow data for the 5 hour experiments are illustrated for trivial, moderate and severe ischemia. With each degree of ischemia, there was no significant change in blood flow between ½, 2½ and 5 hours of the ischemic period.

**Biochemical data (Table 1 and Fig. 3).** The profile of ATP metabolites in nonischemic myocardium was normal. The ATP to adenosine diphosphate (ADP) ratio and adenylate energy charge ([ATP + ½ ADP]/[ATP + ADP + AMP]) (where AMP = adenosine monophosphate) were high, and the tissue contents of the end products of ATP degradation, namely adenosine, inosine and hypoxanthine, were low. With severe ischemia, myocardial ATP content fell to very low levels, consistent with the observations of others (3,10) who used complete coronary artery occlusion to produce comparable decreases in blood flow. The disorder in ATP metabolism was progressive. ATP content was 21 nmol/mg protein (55% of nonischemic control myocardium) after ½ hour and 2.2 nmol/mg protein (6% of control) after 5 hours. The total adenine nucleotide content (sum of ATP + ADP + AMP) of severely ischemic myocardium also fell further between ½ and 5 hours (29 to 6.1 nmol/mg protein). The adenylate energy charge decreased from 0.92 in nonischemic tissue to 0.85 in tissue made severely ischemic for ½ hour and to 0.52 in tissue made severely ischemic for 5 hours. The sum of purine nucleosides and bases,

**Figure 1.** Hemodynamic findings. Symbols represent mean values (± SEM) for ten ½ hour experiments (expts) (left) and for ten 5 hour experiments (right). With left circumflex (LC) artery stenosis, aortic pressure decreased from the baseline value (0 hours) (p < 0.01). Heart rate increased slightly (p < 0.05) in the ½ hour experiments. Blood flow decreased in the myocardium supplied by the stenotic artery. The decrease in flow was proportionately greater in the subendocardium (subendo) (open circles) than in the subepicardium (subepi) (closed circles). There was no significant trend in the intensity of myocardial ischemia in either the subendocardium or subepicardium during the 5 hour period of ischemia.
barely detectable in control nonischemic samples (0.08 nmol/mg protein), increased to 6.2 nmol/mg protein at ½ hour and increased further to 15 nmol/mg protein at 5 hours. Despite this large accumulation of nucleosides and bases, mainly of inosine and hypoxanthine, the sum of ATP and its primary degradative products was depleted by approximately 50% after 5 hours of severe ischemia (from 44 to 21 nmol/mg).

**Moderate ischemia.** The time-dependent changes in the concentrations of adenine nucleotides, nucleosides and bases differed when ischemia was less severe. After ½ hour of moderate ischemia, ATP content was 25 nmol/mg protein (66% of control) and the total adenine nucleotide content was 32 nmol/mg protein (73% of control). The energy charge decreased to 0.89, and the sum of purine nucleosides and bases increased to 3.9 nmol/mg protein. At 5 hours the ATP content was 20 nmol/mg protein, the energy charge was 0.85 and the total adenine nucleotide content was 26 nmol/mg protein, values not significantly different from those at ½ hour. Thus, in contrast to the results observed with severe ischemia, changes in the tissue content of ATP and its metabolites between ½ and 5 hours were small and not statistically significant. During prolonged moderate ischemia, an abnormal but relatively stable state evolved.

**Subendocardium versus subepicardium.** All myocardial samples with severe ischemia were from the subendocardium, whereas subendocardium and subepicardium were approximately equally common among the moderately ischemic samples. To test the possibility that the difference in time-dependent changes in ATP concentration between moderate and severe ischemia was due to the type of tissue rather than the intensity of blood flow deprivation, we examined subendocardial data separately. We found that stabilization during moderate ischemia did apply to the subendocardium: the mean ATP concentration in the subendocardium was 25 nmol/mg protein in the four samples at ½ hour and 23 nmol/mg protein at 5 hours. Viewed from another angle, the mean ATP concentration in the subendocardium was significantly lower after 5 hours of severe ischemia (2 nmol/mg protein) than after 5 hours of moderate ischemia (p < 0.01).

**Trivial ischemia.** In trivial ischemia, abnormalities in regional myocardial blood flow as measured by the microsphere technique were at the limits of the reliability of the method. In all experiments, however, the flowmeter transducer registered a disturbance in left circumflex arterial flow characteristics. Under these conditions, changes in the tissue content of ATP and its metabolites were minor. Even after 5 hours of trivial ischemia, ATP content was close to normal (78% of control), the adenosylate energy charge was unchanged and no significant accumulation of nucleosides and bases was found.

**Discussion**

**ATP metabolism in moderate ischemia.** The results reported here show that moderate ischemia can produce a derangement in adenosine triphosphate (ATP) metabolism that is not rapidly progressive. Nutritional support to the heart that is inadequate to maintain normal ATP metabolism may still be sufficient to allow relative stabilization of the disturbance in ATP metabolism for at least 5 hours. Stability beyond 5 hours was not tested.

The disturbances in ATP metabolism we found at the end of 5 hours of moderate ischemia or ½ hour of severe

---

**Figure 2.** Data are shown during baseline and during left circumflex (LC) artery stenosis for the ten 5 hour experiments. Each experiment contributed a subepicardial and subendocardial sample. The intensity of ischemia did not change significantly during the ischemic period in samples with trivial (∆) (n = 6), moderate (∇) (n = 11) or severe (○) (n = 3) ischemia.

**Figure 3.** Myocardial adenosine triphosphate (ATP) content after left circumflex (LC) coronary stenosis. Symbols represent mean values (± SEM) at ½ hour and 5 hours of trivial, moderate or severe ischemia. At ½ hour the decrease in ATP content from control (0 hours) was roughly proportional to the degree of ischemia. ATP depletion progressed between ½ and 5 hours in severe ischemia (p < 0.01) but stabilized in trivial and moderate ischemia (differences not significant). Symbols as in Figure 2.
ischemia were similar to those previously reported after 15 minutes of complete coronary occlusion (5-7,10). During reperfusion after 15 minutes of complete occlusion, tissue ATP content (5,7) and contractile function (4) returned to normal, but the process required several days. Although we did not study reperfusion in our experiments, by analogy with the brief occlusion model of ischemia, we hypothesize that the metabolic abnormalities caused by 5 hours of moderate ischemia also would be reversed after several days of reperfusion.

One reason for the delay in restoration of ATP content is that ATP resynthesis from basic materials is slow compared with resynthesis using preformed purine bases. The depletion of the total purine pool in ischemic injury occurs primarily by loss of the freely diffusible purine nucleosides and bases, namely, adenosine, inosine and hypoxanthine. With brief coronary artery occlusion, much of the purine loss occurs as accumulated metabolites are flushed from the myocardium during early reperfusion (18). Our results show that purine loss from the myocardium also occurs during the period of low blood flow. After 5 hours of trivial, moderate and severe ischemia, the sum of ATP and its primary metabolites was 82, 68 and 48%, respectively, of nonischemic values. Thus, purine depletion during ischemia increased with the severity of ischemic insult.

### ATP metabolism and contractile function in moderate ischemia.

Matsuzaki et al. (11) measured cardiac function in dogs while coronary blood flow was reduced by subtotal stenosis to 20 to 60% of normal for 5 hours, a condition comparable with our moderate ischemia. With subtotal stenosis, contractile function decreased to an intermediate level between normal and that observed for total occlusion, and remained at that intermediate level for 5 hours. When the stenosis was released after 5 hours, mechanical function promptly improved but required several days to recover completely. Microscopic evidence of permanent myocardial damage was minimal. These results show that moderate ischemia can lead to a state of partial dysfunction that seems to stabilize and to be tolerated without tissue damage for a much longer period than is possible with severe ischemia. The sustained, moderate alteration in ATP metabolism we report here for partial ischemia parallels the sustained partial decrease in contractile function observed by Matsuzaki et al. (11). In the presence of ischemia, reduced ATP utilization due to diminished mechanical work may help to balance the compromised ATP synthesis.
The state of prolonged mild cardiac dysfunction caused by an episode of intense ischemia lasting for several minutes, that is, long enough to deplete ATP but not long enough to cause necrosis, has been termed "stunned" myocardium, and has been proposed as a possible outcome of ischemic attacks in humans (19). On the basis of our results, and those of Matsuzaki et al. (11), we suggest that metabolic and functional "stunning" also occurs when a more moderate degree of ischemia is prolonged for several hours.

Potential period of salvage after coronary reperfusion. Recent development of effective coronary thrombolysis in patients with acute myocardial infarction has focused sharp attention on the question of how long ischemic myocardium can remain viable. The dog model of acute coronary occlusion suggests that damage is irreversible after only a few hours. In humans, however, coronary artery obstruction and partially compensating collateral vessels often evolve together slowly, leading to various degrees of regional ischemia within the heart. The potential period for salvage might be much longer. In patients with acute myocardial infarction, Sobel et al. (20) showed metabolic evidence for viability in the previously ischemic myocardium even when thrombolysis was not achieved until 7 hours after symptoms began. Improvement in systolic cardiac function also may be possible in patients with delayed reperfusion, and the improvement may require a prolonged period. For example, Reduto et al. (21), initiating therapy at an average of 9 hours, reported an increase in left ventricular ejection fraction 10 days later, whereas measurements made immediately after thrombolysis had shown no significant change. Also, patients with subtotal coronary occlusion or angiographically visible coronary collateral vessels more often exhibit later improvement in systolic function, whether they receive streptokinase (22) or routine medical management (23), suggesting that when the intensity of cardiac ischemia is moderated either by residual anterograde perfusion or by partially compensating collateral flow, the period of potential reversibility by reperfusion lengthens, and the ischemic myocardium may even recover spontaneously.

Conclusions. Taken together, these clinical findings suggest that 1) the interval of time during which salvage of myocardium is possible during acute infarction varies from one patient to another according to the intensity of ischemia during the ischemic interval, and 2) the process of reversal of dysfunction occurs slowly over an extended period of time. These conclusions are consistent with our observations that the metabolic derangement in the myocardium induced by a moderate degree of ischemia can stabilize for several hours at a level of abnormality that is potentially reversible.

References

