CMLS, Cell. Mol. Life Sci. 58 (2001) 351–355
1420-682X/01/030351-05 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Review

Some aspects of biochemistry of myocardial infarction

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Received 2 August 2000; received after revision 25 September 2000; accepted 26 September 2000

Abstract. This review deals with biochemical changes in infarcted heart muscle. Two main topics are emphasized: changes in substrate metabolism and in myocardial nitric oxide (NO), and prostacyclin and thromboxane formation. Alterations in glucose metabolism in infarcted heart are discussed with special reference to its myocardial utilization in ischemia. The biochemical basis of the increase in NO and prostanoids and the relationship between the enzyme producing nitric oxide (NOS) and cyclooxygenases (COX) responsible for formation of prostanoids are described. The relevance of these findings to clinical conditions and to angiogenesis in heart muscle are stressed.

Key words. Infarction; myocardium; nitric oxide; prostacyclin; angiogenesis.

Introduction

Progression of science depends on available tools which may be complex or simple. What appears complex today can be primitive tomorrow. Some of the sciences like theoretical physics or mathematics achieve their goals by thought processes alone. In the field of applied sciences, however, goals depend on proof by experimentation. An example is the biochemistry of myocardial infarction, which within the last 50 years has acquired clinical relevance.

Myocardial infarction is a condition in which part of the heart muscle undergoes anatomical and biochemical changes as a result of ischemia (reduction in regional blood supply). Hypoxia (diminished oxygen tension) is the result of ischemia, and many of the biochemical changes observed in the heart muscle are hypoxia related. In contrast to hypoxia, ischemia is accompanied by diminution in oxygen supply together with restriction of substrate availability. This leads to accumulation of metabolic product and to biological changes related to both aerobic and anaerobic pathways. Biochemical, anatomical and molecular changes in heart muscle are interrelated; changes in biochemistry result in anatomical and molecular alterations, which in their turn influence biochemical events. For instance, migration of inflammatory cells into infarcted heart muscle causes characteristic biochemical events such as nitric oxide (NO) production.

Substrate metabolism

Over the last 120 years, techniques concerned with cardiac substrate utilization have used both in vitro and in situ studies. In vitro studies have preferentially used the isolated perfused heart, a technique devised by Langendorff in Germany and by A. Newell Martin of the Johns Hopkins Hospital in Baltimore [1, 2]. This method has in the course of time been only slightly altered [3]. It is still in use today, although questions have arisen concerning its applicability to in situ conditions. Problems exist with the oxygen-carrying capacity and colloid osmotic pressure of the perfusion fluid. In particular, the absence of oxygen-carrying pigments in the perfusate limits the investigation. In some cases,

infarction is produced in situ, and the isolated heart is subsequently studied in vitro. Nevertheless, the perfused heart remains a useful tool in the study of biochemistry of the ischemic myocardium because the experiments can be controlled.

In 1878 Kronecker reported on the nutrition of the perfused frog heart [4]. The frog heart was frequently used at that time by a number of investigators. He reported that the frog or turtle heart will stop beating after a short time if perfused with saline, whereas activity can be sustained for longer periods if blood is being used. Kronecker also believed that the heart could beat without oxygenation of the perfusion fluid, possibly an early demonstration of the potential of anaerobic cardiac metabolism. Locke and Rosenheim found in 1904 that dextrose disappeared when it was perfused through the mammalian heart. They concluded that this was due to a fundamental chemical change underlying cardiac activity, rather than a 'metabolic or fermentation by-process' [5]. Later, C. A. Evans determined mechanical efficiency, resting metabolism and influence of the diastolic volume; he published a graph relating oxygen uses of the heart to the heart volume [6]. In addition, using the heart lung preparation he determined the effect of heart rate to oxygen usage, and reported on myocardial nutrition by determining myocardial utilization of glucose, lactate and pyruvate. He found that myocardial usage of lactate was influenced by oxygen usage. It seemed that carbohydrates were of special value as fuel for the heart [6].

The heart's ability to utilize carbohydrates, amino acids and fatty acids was reported in 1954 [7]. Using the human heart, it was found that myocardial glucose usage and extraction are a function of arterial glucose concentration [8]. This suggested that at low sugar levels, substances other than glucose are utilized and that there is a maximum capacity for the utilization of glucose. Myocardial utilization of lactate was also found to be dependent on its arterial concentration. But total aerobic metabolism of these substrates accounted for less than 35% of the total, suggesting that the heart also utilizes noncarbohydrate material. This was verified when the utilization of free fatty acids was determined in the human heart by means of coronary sinus catheterization. Myocardial usage of free fatty acids was particularly great after high fat intake [9]. It was also observed that in experimental myocardial infarction, glucose extraction rose to values above control within minutes after induction of ischemia, demonstrating increased myocardial glucose utilization in myocardial ischemia [10].

In 1975 Neely and co-workers [3] found an initial acceleration of glycolysis in the isolated rat heart due to a more rapid rate of glycogenolysis. Previously, Wollenberger had demonstrated in situ that if coronary blood flow was arrested, glycolysis was increased [11]. Neely and his associates found that washout of the interstitial space with removal of lactate and maintenance of cellular pH were important factors in maintaining accelerated glycolytic flux. The lack of washout appears to complicate energy production in the ischemic heart muscle [3]. The difference between pure anoxia and hypoxia and ischemia, although of biochemical interest, is less relevant for the heart in situ, where pure myocardial hypoxia is unlikely because of adjustments of the coronary circulation. For this reason, the study of the effect of pure myocardial anoxia or hypoxia has been primarily restricted to the isolated heart perfused in vitro.

The major studies concerning the myocardial usage of glucose in myocardial ischemia originated in the laboratory of Lionel H. Opie. He and his co-workers, found increased removal of glucose from the perfusate in the isolated ischemic perfused rat heart, even though the absolute myocardial glucose uptake was decreased in severe ischemia. They observed that the rate of glycolysis in myocardial ischemia is primarily due to the rate of glucose delivery and subsequent transport into the cell. They did not entirely exclude concomitant enzyme inhibition, but increase of glucose delivery appeared to be of greater importance [12, 13]. Glucose oxidation and glycogen resynthesis during ischemia promote the return of normal contractile function in the postischernic heart [14].

The question arose as to the cause for the increased myocardial glucose utilization in ischemic myocardium. Sun and co-workers [15] found that the major cardiac glucose transporter, GLUT4, is translocated to the plasma membrane after myocardial cells are subjected to acute ischemia. Translocation of rat myocardial glucose transporters by insulin had already been demonstrated [16].

Cardiac fatty acid uptake and transport have been thoroughly described by van der Vusse and co-workers [17]. Apparently, proteins are involved in the uptake and transport of long-chain fatty acids. Membrane proteins are also important for transfer of fatty acids across endothelial and muscle cell membrane. Fatty acids themselves appear to be responsible for the protein interaction. It comes as no surprise that chronic alterations in the cardiac utilization of fatty acids are genetically controlled, involving most phases of myocardial fatty acid metabolism. Exogenous fatty acids can also act in cardiac transcriptional activity [18]. Elevation of free fatty acids in plasma after myocardial infarction is likely due to a surge of catecholamine activity. Elevation of free fatty acids in plasma increases the incidence of ventricular arrhythmias [19]. Free fatty acid concentration increases in plasma are due to inhibition of β oxidation of lipids in mitochondria and due to

accumulation of intracellular acyl carnitine and acyl-coenzyme A (CoA) acyl carnitine. Increased glucose concentration reduces the myocardial uptake of free fatty acids. In a series of papers, Opie has summarized the importance of fatty acids and glucose in myocardial ischemia [13].

Increased glucose uptake by the ischemic heart has been confirmed by positron computed tomography (PCT) scanning. PCT can measure exogenous glucose utilization quantitatively in the myocardium using [^{18}F]-2-fluoro-2-deoxyglucose (FDG), whereas myocardial perfusion is determined by N-13 ammonia [20]. In a majority of patients with coronary heart disease, certain zones of myocardium show discordant increases in FDG activity relative to N-13 ammonia. This discrepancy between the increased uptake of glucose and the fall in coronary blood flow has been called 'metabolism/perfusion mismatch' [21]. This mismatch has been used as an indicator for myocardial viability after myocardial infarction. Obviously, myocardial utilization of glucose signifies viability; on the other hand, when both coronary blood flow and myocardial glucose utilization are diminished, the heart ceases to be viable.

In 1962 Sodi-Pallares and co-workers [22] presented treatment of patients with acute myocardial infarction with a mixture of potassium, glucose and insulin. The rationale was 'if in the dead zone or injury zone of infarction there is a low membrane resting polarization, we are forced to admit that the intracellular potassium is proportionately decreased if K_0^+ remains constant' (K_0^+ represents the concentration of potassium outside the fiber). Sodi-Pallares believed that treatment with glucose, insulin and potassium forces potassium into the cell, thus restoring the normal resting potential. The rationale of this treatment appears primitive by today's standards in the light of the knowledge of ion gating across cardiac cell membranes. However, the overall importance of the use of glucose, insulin and potassium remains. Numerous articles have appeared in the literature testifying to the value of glucose-insulin-potassium treatment in acute myocardial infarction. Opie has summarized those in two editorials [12, 13]. He described a reduction of 28% in mortality with 49 lives saved per thousand patients treated [13]. Other studies published in 1998 [23] summarized results on 407 patients. The most striking results were found in two subgroups of patients—those who had also been reperfused with thrombolytic therapy and those treated with high doses of glucose, insulin and potassium. Even in patients who had already received thrombolytic agents, the in-hospital death rate was reduced by over 60%. Apstein and Taegtmeyer [24] mentioned several factors which delayed the recognition of treatment with GIK. Amongst them are the fear that the treatment may worsen myocardial acidosis as a result of increased lactate production, and failure of the pharmaceutical industry, which did not want to sponsor research on therapy without the possibility of patents and profits.

It is therefore certain that glucose-insulin-potassium infusion still has a future in the treatment of severe acute myocardial infarction as well as an adjunct to treatment by thrombolysis and angioplasty.

Myocardial ischemia, NO and prostanoid production in infarcted heart muscle

In recent years, the discovery of increased NO, prostacyclin and thromboxane formation in infarcted myocardium has added a new dimension to the understanding of myocardial ischemia. Production of NO favorably influences contractile and metabolic functions of the infarcted heart [25–27]. In contrast, in solid tumors, NO leads to angiogenesis and spread of the tumor. Nitric oxide in infarcted heart muscle is released primarily through the enzyme action of the inducible form of NO synthase (iNOS) by activated macrophages [28, 29]. By binding to the iron in heme group of guanylate cyclase, NO produces cyclic guanosine monophosphate (cGMP), which activates a cascade of cellular processes responsible for its physiological and pharmacological effects [30]. Under physiological conditions, the activity of iNOS is relatively low but increases following myocardial ischemia, primarily through activation of invading macrophages by cytokines [28]. Stuehr first recognized that NO_2 (nitrite) and NO_3 (nitrate) are the inactive end-products of NO [31]. It was later found that these oxidation products of NO are increased in the blood of animals with experimental myocardial infarction and in patients following coronary artery occlusion [32, 33]. This may be important for cardiac activity since NO counteracts myocardial thromboxane formation, inhibits platelet activation, stimulates synthesis of prostanoids through activation of COX and reduces myocardial contractility; it activates formation of cGMP. Other effects of NO are dilation of coronary arteries, suppression of ventricular fibrillation and reduction in infarct size [34]. NO also reduces myocardial oxygen consumption without affecting contractile efficiency [35]. Peroxynitrite, a NO metabolite, plays a particular role in reperfusion of ischemic myocardium [36]. Peroxynitrite as well as hydroxy-like intermediates are present at critical times during postischemic injury and may also act as mediators of biological injury. It is likely that only exogenously supplied NO protects the heart against the damage of ischemic reperfusion [37].

An important feature of myocardial NO is the relationship between activation of NO synthase (NOS) and

cyclooxygenase (COX), the enzyme that forms prostanoids from arachidonic acid. NO, together with prostacyclin and thromboxane, is produced in the infarcted heart muscle in situ [38] and by microsomes obtained from infarcted dog hearts [39]. It appears that NO released exogenously from clinically used NO donors activates COX, leading to the release of prostacyclin [40]. Additionally, the inflammatory cytokine interleukin-1β induced both COX and NOS by mesangial and osteoblastic cells [41, 42]. However, there are still questions concerning the cross-talk between NO and COX systems. In infarcted heart muscle, a rise in production of NO is accompanied by an increase of prostacyclin and thromboxane.

Prostacyclin and thromboxane synthesis are increased in infarcted heart muscle [34]. Prostacyclin inhibits platelet aggregation, causes coronary vasodilation, prevents ventricular arrhythmias and decreases infarct size [39]. Thromboxane, on the other hand, promotes platelet aggregation, causes vasoconstriction, initiates ventricular arrhythmias and increases infarct size [39]. Increased production of NO and prostacyclin counteracts these effects.

Nonsteroidal antiinflammatory compounds, such as acetylsalicylic (aspirin)influence NO, prostacyclin and thromboxane production in infarcted myocardium. Aspirin, a COX1 and -2 inhibitor, reduces both prostaglandin and thromboxane formation. Seventy-five milligrams per kilogram per day of aspirin diminishes concentrations of prostacyclin and thromboxane in infarcted heart muscle but fails to influence the myocardial activity of iNOS [38]. The selective COX2 inhibitor, celecoxib, also lowers myocardial prostacyclin production. However, it does not interfere with myocardial induction of iNOS and therefore does not alter the myocardial production of nitrite and nitrate [43].

NO and prostanoids appear to play a role in the initiation and development of angiogenesis in infarcted heart muscle. There is considerable evidence that angiogenesis and NO are interrelated. Some of this information has been collected from a study of NO production in solid tumors [44, 45]. Here, NO synthase and cGMP are significantly higher than in normal tissue. However, whereas in solid tumors production of NO and prostanoids is undesirable because they promote growth of tumor and its spread, in myocardial infarction NO and prostanoids are advantageous because of the initiation of new vascular growth. The production of cytokines by inflammatory cells with subsequent activation of iNOS and COX occurs both in infarcted myocardium and in solid tumors [46].

The relationship between NO and angiogenesis has been repeatedly confirmed in infarcted myocardium. NO donors and growth factors induce angiogenesis in vivo and cause proliferation of endothelial cells in cell cultures [47–49]. NO production appears to be necessary for the growth-promoting effect of vascular endothelial growth factor (VEGF). These findings may have considerable implications for the use of NO donors as anglogenesis-promoting factors in the ischemic myocardium.

Molecular biology is now beginning to contribute to the events occurring in infarcted heart muscle. Although this review does not concern itself with these molecular processes, reference is made to several articles in which the importance of molecular biology and genetics in myocardial infarction are discussed [50–53].

One of the triumphs of modern medicine has been the reduction in deaths resulting from myocardial infarction. This has been primarily the result of mechanical procedures (coronary angioplasty, stenting) and of advanced knowledge in mechanisms of blood clotting. Yet knowledge of biological changes in infarcted heart muscle has greatly contributed to understanding of a disease which still rates as the number one cause of death in Western society.

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