

Biochemical basis to block ischaemia developing into myocardial infarction : a short review

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Abstract

The altered myocardial metabolism after an ischaemia is one of the recently recognized fields of interest. The molecular events involved from ischaemia to cell necrosis are still very controversial although, it seems that the overall metabolic changes have profound ill-effects on myocardium. It has also become apparent that if one could effectively reduce tissue damage following ischaemia, its overall consequences might be averted. Many potential therapies designed to protect the ischaemic heart improve energy metabolism through changes in metabolic pathways. This article reviews some of the latest experimental findings and summarizes the present knowledge concerning biochemical basis of prevention of cellular injury in clinical and experimental myocardial infarction.

Key words : Myocardial metabolism, myocardial infarction, energy metabolism, potential therapies.

1. Introduction

Myocardial infarction has become one of the leading causes of morbidity and mortality in most of the countries. During the last decade attempts have been made to reduce the injury to ischaemic myocardium by the use of newer therapeutic agents. Alteration in cardiac metabolism has been implicated as a cause of cardiac injury and a better understanding of cardiac metabolism in health and ischaemic heart disease is essential for developing metabolic intervention to ameliorate further damage to ischaemic myocardium. One of the most formidable barriers to the clinical application of the information which has been obtained in the laboratory is the lack of a suitable technique for assessing the efficacy, or lack thereof, of these interventions. It seems desirable, therefore, that an intensive research effort should be directed towards ascertaining the definitive clinical value of therapies aimed at the protection of the acutely ischaemic myocardium. This review first considers the possible initial events in developing myocardial infarction and later, the factors promoting the progression from ischaemia to infarction along with the effects of therapeutic agents are considered.

2. Ischaemia to infarction

The difference between myocardial ischaemia and infarction is that whereas the former is a reversible decrease in blood flow severe enough to have metabolic consequences such as depletion of ATP and anaerobic metabolism²³, the latter is the irreversible development of tissue necrosis. According to Opie⁴⁴, factors concerned with the initiation of ischaemic injury are: (1) vascular factors, including the role of atheromatous plaque, (2) haemodynamic factors such as the imbalance between the supply and demand of oxygen in the ischaemic zone, (3) catecholamine activity, which is generally harmful to the developing infarct. Although many aetiological factors participating for such injury, it is believed that the metabolic events may be responsible for the progression of ischaemia to infarction (Fig. 1). Moreover from the clinical point of view, enzyme release from the heart is virtually synonymous with cell necrosis and constitutes a diagnostic aid used by cardiologists. We are still lacking with the very basis of the molecular mechanisms involved here¹⁴, and identification of these would be extremely important.

Two of the features of acute myocardial infarction which are of major clinical importance are, first, the mechanism of myocardial cell death, and secondly, the development of serious ventricular arrhythmia. The principal initial events in ischaemia are deprivation of the supply of oxygen and nutrients and inability to remove products of cell metabolism⁴. Induction of ischaemia or anoxia is followed, virtually instantaneously, by a complex series of events, involving every organelle and subcellular system, occasioned by the acute deprivation of oxygen and ATP production. This involves changes in protein⁶⁶ and nucleic acid synthesis²⁷, changes in ion transport across plasma membrane¹⁹, cessation of fatty acid oxidation⁶², glycolytic inhibition⁶⁷ and ultimately, autolytic events related to the hydrolases⁶⁴ present in various compartments. It is important to define these sequence of events⁵⁴ and especially to determine the crucial event not only for better understanding but also for the development of interventions that might modify the course of cell damage and the vicious cycle (Fig. 2) in myocardial infarction.

3. Glycolytic inhibition

The myocardium provides itself with a constant supply of high energy phosphate, primarily through oxidative pathways under aerobic conditions. Normally, the utilization of carbohydrate and lipid in heart muscle is closely coupled to the energy needs of the heart. When the substrates are present together, fatty acid is used in preference to glucose⁴². The rate of fatty acid uptake and oxidation by aerobic heart muscle is controlled primarily by the rate of acetyl-CoA oxidation by the citric acid cycle.

It is unlikely that ATP production by substrate level phosphorylation in glycolysis is ever of major quantitative importance in aerobic hearts and can compensate only a negligible per cent of the loss of oxidative production of ATP in anoxia or ischaemic hearts. Thus, Kobayashi and Neely²⁶ have suggested that the quantitative importance

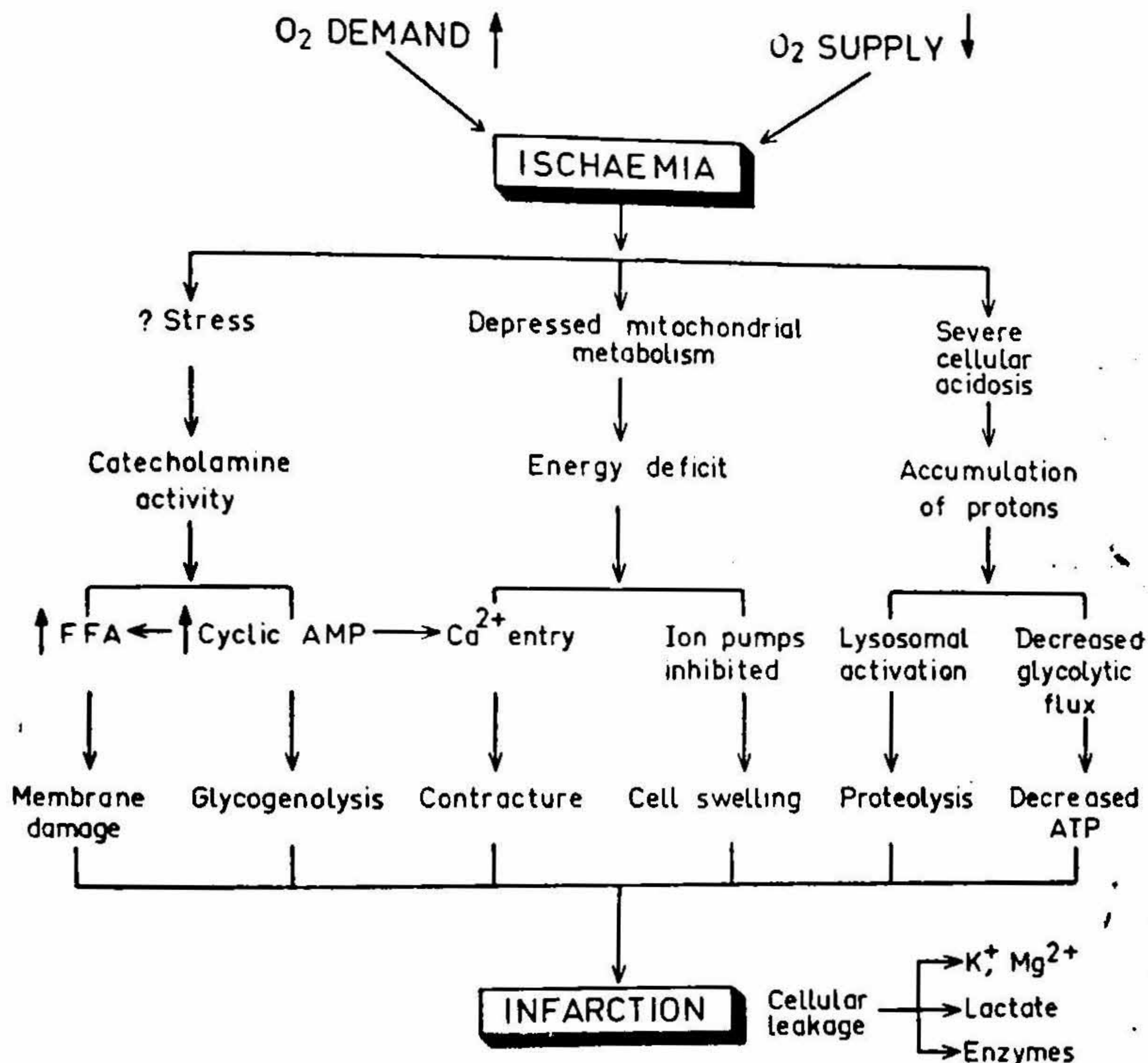


FIG. 1. A schematic representation of the cellular events concerned during the change from ischaemia to infarction. The onset of cellular leakage is still controversial and needs further investigation. The overall biochemical disturbances reflect the very basis of prevention of cellular injury in myocardial infarction.

of glycolysis in energy metabolism is not ATP synthesis, but rather the production of pyruvate which is subsequently oxidized by citric acid cycle. Normally, the low contribution of glycolysis to ATP production is due to inhibition of phosphofruktokinase (PFK) by free-fatty acids, high tissue levels of inhibitors such as ATP, creatine phosphate (CP) and citrate and low levels of activators such as ADP, AMP and fructose-1, 6-diphosphate (FDP). In fact, PFK is the rate controlling step of glycolysis in heart similar to other tissues⁵⁸ under most physiological condition and acceleration of this step due to low level of inhibitors accounts for faster rates of glycolysis in oxygen-deficient tissues,

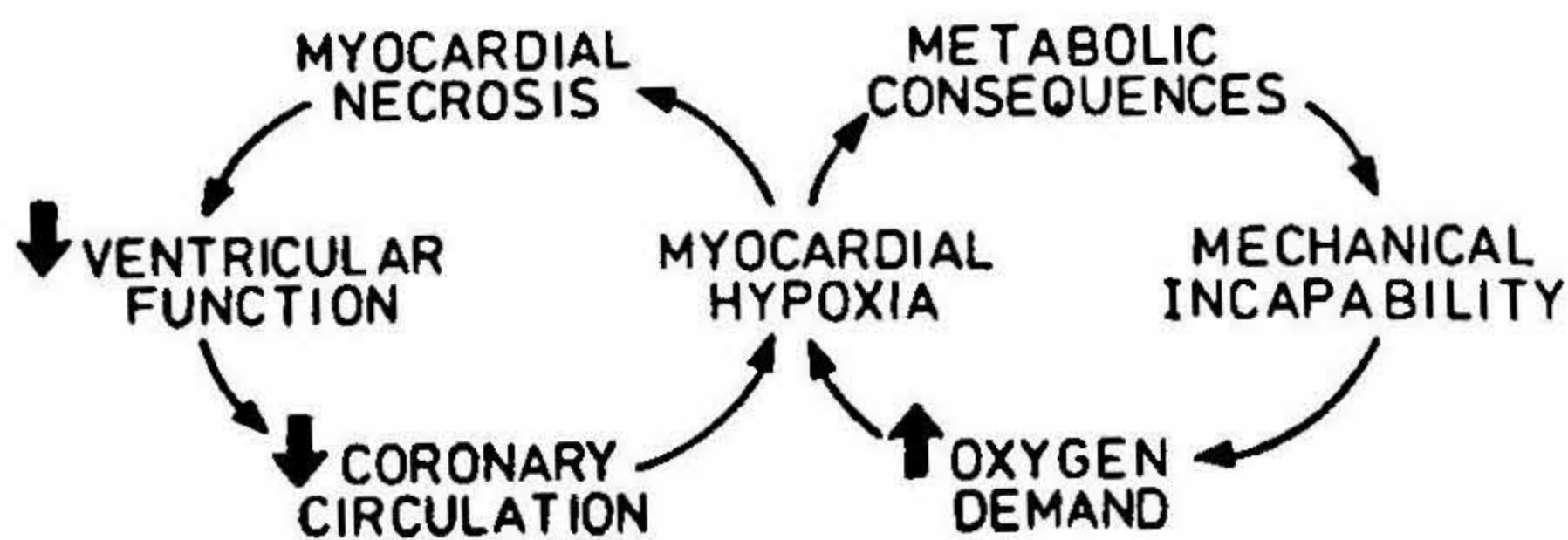


FIG. 2. Scheme to show the multiple factors participating in the vicious cycle of myocardial infarction.

The most important criterion raised and experimentally documented is that the Embden-Meyerhof pathway catabolic activity is not increased, but rather impaired by the progressive inactivation of several enzymes in the course of acute myocardial ischaemia. A critical step in ischaemic inhibition of glycolysis appears to be inactivation of PFK induced by progressively increasing intracellular acidosis, thus depriving the pathway of FDP. One approach to relieve this would be to provide FDP exogenously. Such an increase in the cytoplasmic concentration of FDP should stimulate glycolysis by intervening as a metabolic regulator and substrate to permit function at higher rates. Markov *et al*³¹ have shown that the intravenous administration of FDP has restored the depressed activity of glycolysis in ischaemic myocardium.

Interestingly, Rovetto *et al*⁴⁸ have noted in experimental animals that induction of ischaemia results in a transient increase of glycolytic flux which is followed by inhibition; moreover, PFK is inhibited in severe ischaemic condition and the slowest step in the glycolytic pathway is shifted to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). This conclusion is supported by the observation that acceleration of PFK in ischaemic hearts results in increased tissue levels of FDP, dihydroxy acetone phosphate and glyceraldehyde-3-phosphate^{48, 55}. The increased tissue lactate concentration, NADH/NAD⁺ ratio and decreased pH may be important factors for these inhibitions (Fig. 3). Oxidation of cytosolic NADH in ischaemic hearts is complicated by slow coronary flow and lack of washout of cellular lactate, which affects the other pH sensitive cellular activities (Table I). On the basis of computer-stimulated data, Kohn and Garfinkel²⁸ have recently shown that there is a lactate permease, which is normally activated by protons but during ischaemia it is inhibited. To decrease lactate from ischaemic tissue it is more logical to increase the activity of lactate permease by pharmacological intervention.

Since both lactate and H⁺, in the presence of oxygen deficiency, lead to an inhibition of glycolysis, attempts to improve the glycolytic rate are centred around these two variables. Maintenance of pH does enhance glycolysis in ischaemic hearts⁶³. The higher pH increases glycolysis by the following two mechanisms: The major effect of lower H⁺ concentration appears to be on the coronary vascular resistance which is

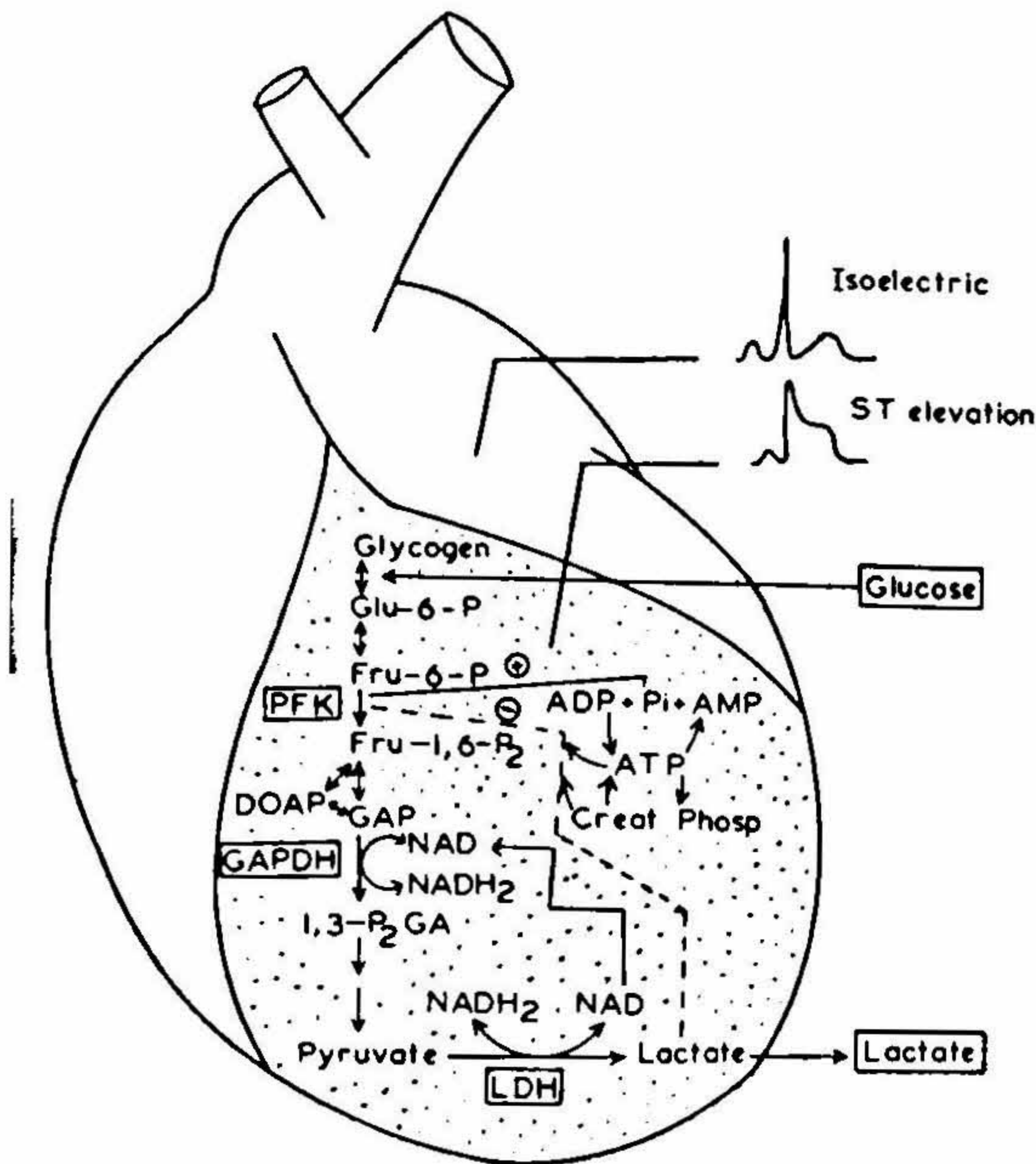


FIG. 3. A schematic illustration of the effect of ischaemia on myocardial glycolytic flux. Glycogen is broken down and subsequently, intracellular accumulation of proton leads to inhibition of glycolytic flux through phosphofructokinase (PFK) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), in spite of the decrease of tissue levels of creatine phosphate (CP) and adenosine triphosphate (ATP). ST elevation on ECG reflects damage in ischaemic zone.

reduced in the presence of extra buffer. The second effect seems to be due to increased flux of glucose through PFK and GAPDH.

4. Prevention of cellular injury

4.1. Glucose-insulin-potassium (GIK)

Sodi-Pallares⁵² has suggested that increased rates of glucose metabolism and glycolysis may help to promote survival of the oxygen-limited myocardium and infusions of GIK were able to increase cardiac glycogen in the early stages of myocardial infarction. Nevertheless, increased glycogen stores are known to protect against myocardial ischaemic damage¹⁶. As judged by multiple biopsy samples, GIK caused relatively small changes in lactate, with more definite increase in glycogen in the infarct zone⁵⁹. These changes do not prove increased anaerobic glycolysis during GIK infusion. However, it is possible that increased aerobic glycolysis could be involved in the maintenance

Table I

pH sensitive cellular activities

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- (a) Phosphofructokinase⁸¹
 - (b) Glyceraldehyde-3-phosphate dehydrogenase⁴⁸
 - (c) Calcium ion movements and contraction⁵⁸
 - (d) Phosphodiesterase activity⁴⁸
 - (e) Cyclic AMP-dependent phosphorylation of troponin⁸⁷
 - (f) Lysosomal enzymes⁸
 - (g) Buffering capacity of bicarbonate⁶³
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of membrane integrity with decreased ischaemic damage. Another fate of the glucose extracted could be an increased provision of α -glycerophosphate for intracellular esterification of tissue-free fatty acids. Though it is not conclusively known how glucose brings down fatty acids, it is possible, as has been reported by Neely *et al*³⁸ that increased myocardial performance might improve tissue perfusion, decrease catecholamine levels and thereby inhibit lipolysis. The association between increased glucose and decreased free fatty acid in heart with simultaneous tissue metabolic improvement after GIK suggests that it might be beneficial in patients with acute myocardial infarction. In fact, there are reports where people found a beneficial effect of GIK in patients with acute myocardial infarction, as judged both by mortality and by enzyme release⁶.

4.2. Antilipolytic agents

As discussed earlier, free fatty acids (FFA) are established as the major fuel for the fasting heart; they inhibit glycolysis at several levels; hence increase cardiac glycogen content. The new emphasis is given on the toxic effects¹ at high FFA levels. Gupta *et al*¹⁹ reported that acute myocardial infarction is accompanied by an early rise of FFA in plasma, probably due to increased adipose tissue lipolysis as a result of enhanced sympatho-adrenal activity. Those subjects with the highest plasma FFA concentrations have been considered to be at the greatest risk of developing serious ventricular arrhythmias⁴⁰ and death. Such observations have raised the possibility of limiting the ill-effects by measures which decrease the delivery of FFA to the ischaemic cells.

Antilipolytic agents basically inhibit catecholamine-induced lipolysis in adipose tissue with a fall in plasma concentrations and myocardial uptake of FFA. Antilipolytic agents like β -pyridylcarbinol (nicotinic acid derivative), *p*-chlorophenoxyisobutyrate (CPIB), prostaglandin-E₁ (PGE₁) have been effective in modifying infarct size^{8, 12}. Inhibition of lipolysis may offer considerable protection on myocardium. From a clinical point of view it is of particular interest that β -pyridylcarbinol does not interfere with the contractility of the nonischaemic myocardium and therefore Kjekshus²⁶ found that it

can be used without much risk in patients with cardiac decompensation. Tansey *et al*⁶⁰ have demonstrated that oral sucrose is also effective in increasing the rate of fall of plasma FFA from peak levels in patients with smaller infarcts. Since sucrose is unlikely to have any side effects, further studies should be undertaken to see whether repeated sucrose administration would prevent the FFA rebound.

4.3. Role of carnitine

Fritz and Kaplan¹³ have considered that the intracellular accumulation of an intermediate of fatty acid metabolism, acyl CoA impaired mitochondrial activity in myocardial infarction. These observations have focussed attention on the clinical importance of a better understanding of fatty acid metabolism. Fatty acids taken up by the heart must first be activated to fatty acyl CoA before their further metabolism. For their oxidation inside the mitochondria this activated long chain fatty acid combines with a carrier, carnitine (3-hydroxy-4-trimethyl ammonium butyrate) to form fatty acyl carnitine, which crosses the mitochondrial membrane inwards. During ischaemia, acyl CoA accumulates²¹ and impedes mitochondrial synthesis and transfers ATP to the cytoplasm when it is needed for contraction (Fig. 4). In fact, in experimental ischaemia, tissue carnitine decreases⁵¹ and may become rate limiting for transport function. Brooks *et al*⁷ have observed that the infusion of carnitine restores the defect and decreases the ischaemic damage.

It is of great importance to know whether carnitine availability is ever likely to be limiting in ischaemia, and/or whether provision of carnitine could accelerate removal of acyl CoA and thereby improve ischaemia. The most convincing argument for the possible therapeutic use of carnitine would depend on the projected role of acyl CoA impairing mitochondrial metabolism during ischaemic damage. Prior animal experiments should be directed to confirmation of existing reports that there is a correctable deficiency of carnitine which develops in ischaemia. The biochemical basis of this new approach to acute myocardial infarction holds exciting possibilities of the future.

4.4. Steroids and lysosomal protection

For many years, little interest was shown in lysosomes of cardiac muscle. Lysosomes are small vesicles of digestive enzymes which "bud off" the Golgi apparatus. These enzymes are acid phosphatase, acid ribonuclease, acid deoxyribonuclease, acid beta-glucuronidase, cathepsin and catalase. A number of questions are posed by the lysosomal enzyme studies, some accessible to further study and some related to our understanding of the cell. It has been discussed earlier that ischaemia produces lowered ATP, lowered pH and accumulation of membrane-active fatty acids. Romeo *et al*⁴⁷ have suggested that these events, in turn, produce a reduction in membrane integrity. As a result of that, lysosomal enzymes "leak out" of lysosomes. This results in abnormal breakdown of cellular constituents because of (a) abnormal contact between

hydrolytic enzymes and potential substrates, and (b) the presence during ischaemia of a decreased pH⁶⁴. If it is severe enough, this abnormal hydrolysis of cellular constituents could contribute to the development of cell damage (Fig. 4) and, ultimately necrosis. Pharmacological intervention has been tried with methyl prednisolone for its membrane stabilizing property^{1, 18}. Probably it stabilizes lysosomal membrane and prevents leak out of lysosomal enzymes and thus, protects against autolytic and heterolytic damage.

4.5. Hyperosmotic mannitol

Until recently, the regulation of intracellular fluid volume in disease states has received little attention. The principles of the regulation of intracellular volume have been well established. It is suggested that the cell has a high protein content that exerts an osmotic pressure and tends to draw fluid, primarily the salts of sodium from the extracellular compartment into the intracellular space. In healthy tissue, swelling of cells does not occur due to the active and continuous extrusion of sodium from cells by the "sodium

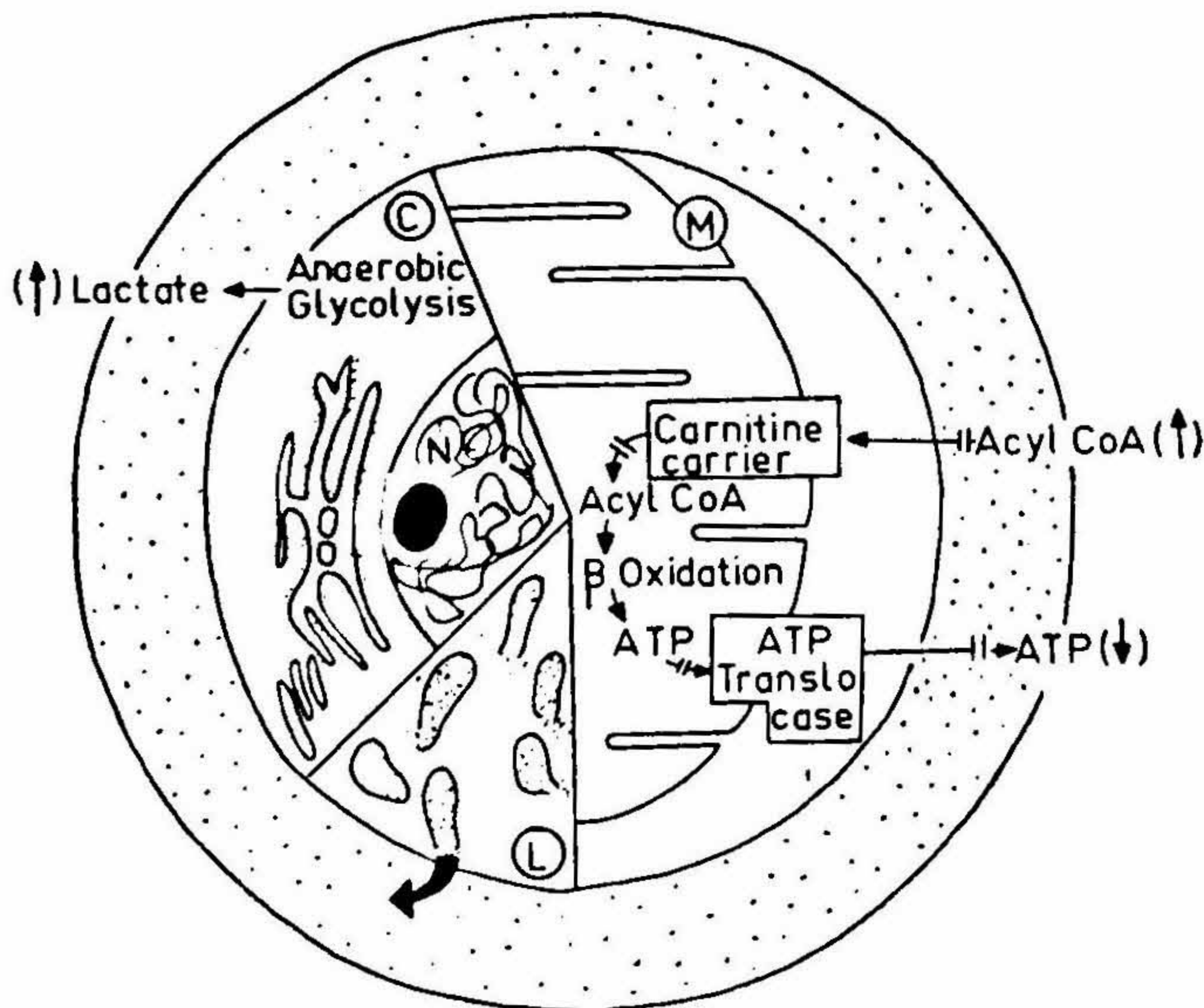


FIG. 4. Simplified version of the effect of ischaemia on carnitine carrier system and on lysosomal (L) membrane within the cell. In ischaemia mitochondrial (M) β -oxidation of activated fatty acid (acyl CoA) is defective as a result acyl CoA accumulates and the production of ATP is limited. Simultaneous accumulation of lactate from cytoplasmic (C) anaerobic glycolysis changes lysosomal membrane integrity. Lysosomal enzymes, in turn, contribute to the development of further cell damage (shown as outer circle). N—nucleus.

pump" located in the outer plasma membrane. Thus, sodium is constantly pumped out by active, energy requiring transport mechanism. The mechanism by which myocardial necrosis is prevented by the use of hyperosmotic mannitol⁶⁵, is now under investigation. Maroko *et al*³² observed that in the sequence of cellular injury there is cell swelling which is followed by progressive cell damage and the administration of hyperosmotic mannitol markedly reduces the degree of myocardial cell swelling during ischaemia. In addition Ganote *et al*¹⁷ have found that hyperosmolar solution of polyethylene glycol also prevents cell swelling and anoxic enzyme release in perfused hearts. Thus, their influences in carefully selected clinical settings deserve evaluation in the future.

4.6. Hyaluronidase

It is believed that administration of hyaluronidase favours transport of energy-producing nutrients from the blood stream to the myocardial cells⁴¹. Thus, in terms of effectiveness hyaluronidase compares favourably with other interventions such as propranolol (β -blocker), glucose and glucose-insulin-potassium³³ in reducing myocardial necrosis. Though its mechanism of action is open to speculation, hyaluronidase was shown to protect the myocardium from undergoing extensive ischaemic injury and consequent necrosis following coronary occlusion.

Hyaluronidase has been carefully studied in a wide range of experimental preparations and has been shown to normalise ECG patterns in acute myocardial infarction. Moreover, no undesirable side effects have been reported except for allergy. This therapeutic agent also increases coronary collateral flow, prevents the reduction of coronary flow induced by ischaemia, and counteracts the formation of oedema fluid as ischaemia progresses. It would seem, therefore, that the combinations of hyaluronidase with other agents warrants experimental assessment.

4.7. β -Blockers

β -Adrenergic blocking drugs not only mark the most significant advance made in the treatment of cardiovascular disorders during the past decade, but also, broadly speaking, constitute one of the few active therapeutic principles currently available for use in cardiac ailments. These drugs (1) reduce myocardial oxygen consumption, (2) antagonise local and systemic release of catecholamines, (3) have anti-arrhythmic properties and (4) influence myocardial energy metabolism and reduce infarct size.

Propranolol, one of the important β -blockers, has been extensively studied recently. Propranolol increases utilization of glucose relative to that of FFA, increases tissue glycogen and leads to conservation of the available oxygen supply. It is also able to reduce LDH release from myocardium and increases significantly the level of ATP, CP and glycogen in the infarcted cardiac muscle^{16, 43}. Whatever their mechanism of action may be, the β -blockers do serve to protect the myocardium against any sudden increase in sympathetic activity, thereby limiting the consequences of ischaemia and reducing the

risk of focal myocardial necrosis. It might thus be assumed that there can be little else to say about the therapeutic value of β -blocking drugs in patients with coronary heart disease.

4.8. Calcium antagonists

It is generally recognized that Ca^{2+} enters the cell during the slow inward calcium current of the plateau phase of the cardiac action potential. In myocardial infarction the increased Ca^{2+} entry combined with further Ca^{2+} release from intracellular storage sites causes a transient increase of intracellular Ca^{2+} , which promotes contraction by an increase of calcium binding to a troponin subunit in the actomyosin complex. Efflux of Ca^{2+} from the cell occurs, partly in exchange with Na^+ and partly by an energy-dependent process³⁰. It would appear probable, therefore, that effects of ischaemia on contractile function of the heart are mediated directly or indirectly by Ca^{2+} . Nevertheless, it remains an attractive possibility that the decreased mechanical performance of ischaemic myocardium is a secondary consequence of intracellular acidosis, which according to Shen and Jennings⁵⁰ directly affects Ca^{2+} entry during the action potential of the cardiac calcium cycle. The entry of Ca^{2+} into the injured cell is associated with massive loading of mitochondria with calcium phosphate, a phenomenon which interferes with mitochondrial function^{15, 24}. The molecular mechanism of action of Ca-antagonists is based on an inhibition of the trans-membrane Ca^{2+} influx (Fig. 5) into the excited myocardial fibres so that, in the contractile system, less phosphate-bond energy is transformed into mechanical work by the Ca-dependent myofibrillar ATP-ase. Moreover, like propranolol⁴⁶, they may reduce energy expenditure by decreasing contractility, and like dipyridamole, oxyfedrine or nitrate improve energy delivery by increasing coronary flow^{3, 25}. Accordingly, from a point of view of cell energetics, calcium antagonists would appear to be particularly promising for the treatment of severe myocardial ischaemia^{16, 49}.

4.9. Protective effects of electrolytes

As discussed earlier the transmembrane equilibrium of electrolytes plays an important role in normal myocardial function, since K^+ , Na^+ and Mg^{2+} in particular are basically involved in electrical stimulus formation and conduction, as well as myocardial contractility requires the participation of Ca ions. Lehr *et al*²⁹ have suggested that damage to the myocardial cell encompasses the obligatory loss of Mg^{2+} and, if severe enough, also of K^+ , as well as the entry of extracellular Na^+ . Conversely, Mishra³⁷ has proposed that a primary K^+ or Mg^{2+} deficiency may cause myocardial cell injury and necrosis. It is our view that the changes in the tissue electrolyte content in response to myocardial injury are not merely a consequence of cellular impairment and death, but also may play a contributory role in the initiation of cellular dysfunction. The treatment of cardiac disease with "polarizing" electrolyte solutions emphasize that the use of K^+ and Mg^{2+} salts may aid the failing heart, may limit the spread of myocardial necrosis, and may restore partially damaged cardiac cells to full function³⁰.

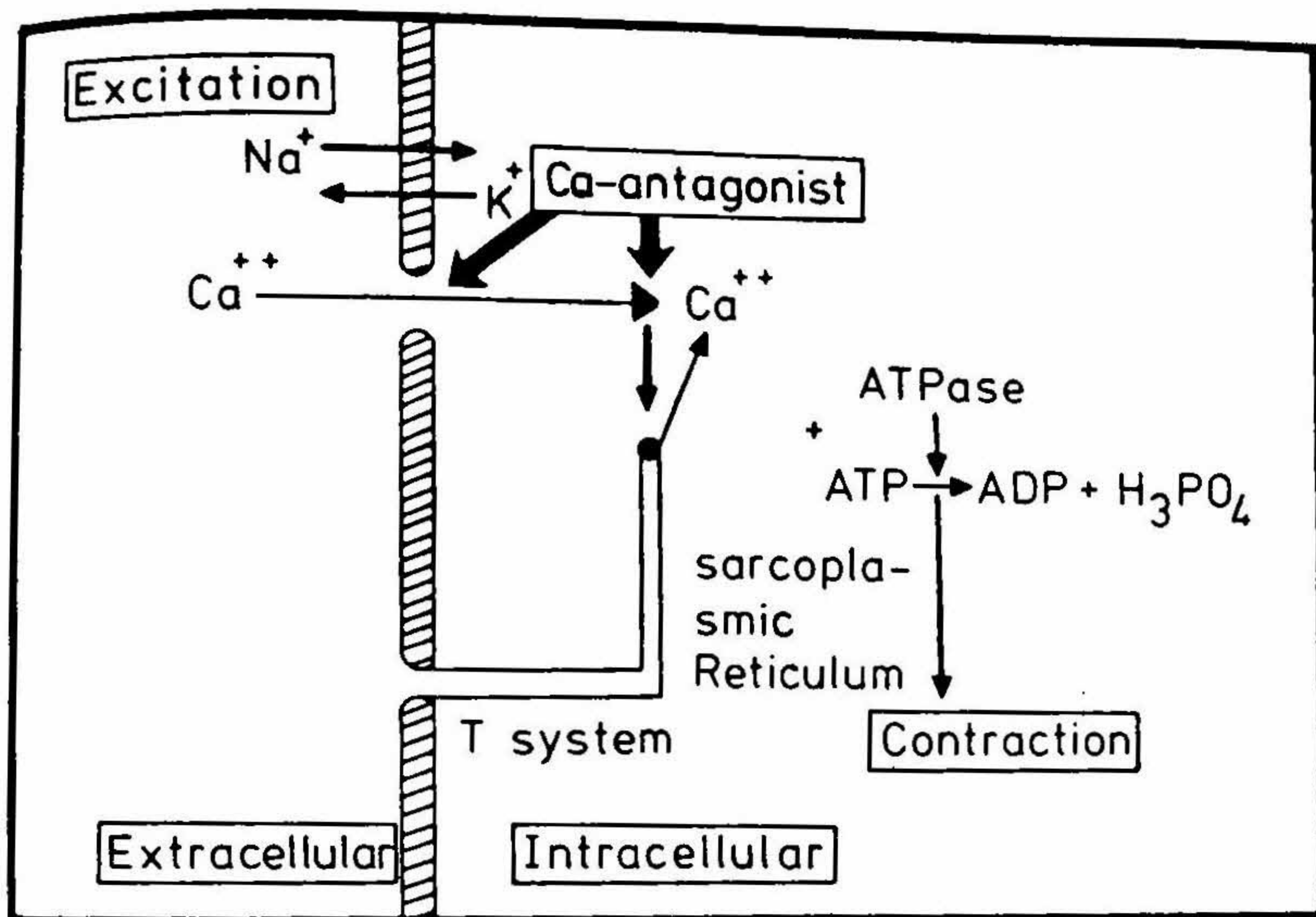


FIG. 5. Proposed site of action of calcium-antagonists on the excitation-contraction coupling in myocardium (After Kroneberg).

4.10. Other biochemical factors

A number of hemodynamic, pharmacologic and metabolic interventions were found to change the extent of acute ischaemic injury of the myocardium and subsequent necrosis following experimental coronary artery occlusions. We have listed some of the promising therapeutic agents (Table II), but firm evidence for their mechanism of action is still lacking. Understanding of cardiac function in a biochemical perspective indicates a series of metabolic defects that reflect the need of the meticulous correction from every angle and special attention should be given to the consequences of cellular process in myocardial ischaemia.

5. Conclusions

Myocardial infarction appears to cause a grave metabolic situation in the heart. Metabolic patterns in cells in zones of developing infarction may, therefore, be extremely complex and heterogeneous. The situation is sufficiently intriguing to suggest that beneficial effects of maximal rates of glycolytic flow could be achieved in ischaemic tissue. It is perhaps true that over the past decade or so, more investigative work has been done

Table II
Potential therapies in myocardial infarction

Intervention with agents	Possible effect on myocardium
Ascorbic acid ^{20,26}	Enhanced recovery process
Aprotinin (inhibitor of Kallikrein system ¹¹)	Decreased leukotactic process, capillary permeability and proteolytic activity
Cobra venom factor ²⁴	Enzymatically cleaves C ₃ of complement system—decreased activation <i>via</i> alternate pathway
Citrate cycle intermediates ²⁸	Stimulate mitochondrial ATP production
Cyclic AMP inhibitors (β -adrenergic blocking agents ²⁶)	Prevents development of cardiac necrosis and arrhythmia
Nicotinamide ³	Inhibits NAD glycohydrolase and is a precursor of NAD ⁺
Taurine (sulfur containing amino acid ⁹)	Enhances functional capacity
Tetrodotoxin ²¹	Selective blockade of Na ⁺ inflow

in the search for a universally successful treatment of ischaemic heart disease and in addition to reducing oxygen requirements *via* alterations in mechanical performance other treatments using different modalities to salvage ischaemic myocardium to limit cellular injury, are currently being tested. All such therapeutic implications would require additional rigorous experimental and laboratory investigation and, where indicated, further clinical trials.

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