

Metabolic Approach of Myocardial Injury During Cold Cardiac Arrest

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Abstract

The goal of this study was to approach the pathological, histochemical and metabolic changes due to the cold ischemic cardiac arrest with crystalloid cardioplegia (Saint Thomas I) during cardiac surgery with cardiopulmonary bypass (CPB).

Method

Four groups of patients were studied during the CPB before aortic cross clamping and after the release of the clamp. Arterial and venous systemic and coronary sinus blood samples were analyzed comparatively. Biopsies of the right atrial appendage allowed electron microscopic and histo-chemical studies, radioimmunological assay of c-AMP, determination of the density of beta-adrenoreceptors (by radio-labeled ligand) and catecholamines induced fluorescence (formaldehyde).

Results

After the release of the aortic cross clamp, we observed a significant decrease of the pH in the coronary sinus comparatively to systemic venous blood and a higher level of lactate excretion rate. Electron microscopic studies showed a transient mitochondrial swelling with a reduction of the glycogene storage in the cytoplasm. In spite of this ischaemia, we did not observe significant variations in beta-adrenoreceptors or c-AMP concentrations. The induced fluorescence of the endogene catecholamines showed a post ischaemic decrease of peri nuclear dopamine store and a diffusion of norepinephrine in the intercellular space as intense as a response of a sympathetic stimulation.

Conclusions

The cold cardiac arrest in spite of the use of the crystalloid cardioplegia induced an anaerobic injury with transient ultrastructural changes and sympathetic-like stimulation but does not alter the main biochemical structures (beta-adrenoreceptors and c-AMP).

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We studied the metabolic and ultra structural modifications of the myocardium during cardiac surgery under CPB with hyperkaliemic cold cardiac arrest. Is the anaerobic cardiac metabolism deleterious in regard to the infrastructure of the myocardial cell and complex biochemical factors such as c-AMP, beta-adrenoreceptors and intracellular distribution of catecholamines?

Materials and Methods

We studied four series of operated patients under CPB with cold cardioplegic arrest. In all cases the CPB was performed with moderate general hypothermia (27°C). The cardiac arrest was coronary infusion of St. Thomas crystalloid cardioplegia (4°C).

In all cases the total CPB was initiated between the ascending aorta for arterial line and the two vena cava with selective cannulation.

Group I - Study of the ischemic anaerobic changes

In this first group of 30 patients (24 males and six females with an average age of 58 years (range 23 to 79), the main goal of the study was to appreciate the acido-basic variations during and after the aortic cross clamping. Twelve patients underwent valve replacement (VR) and 18 coronary artery bypass grafts (CABG). After bypass, two blood samples were collected in arterial and venous systemic blood and in the venous coronary sinus. The first group of sample was collected during the mechanical assistance of the beating heart, the second one just after the release of the aortic clamp (wash out of the ischemic coronary bed).

We performed gas blood and lactate determination with correction in relation to the thermic level. For every catabolite, the systemic and coronary extraction rate was calculated ($A-V/A \times 100$) and the variations between the two steps of the procedure.

Biopsies of the right atrial appendage were made to study by electron microscopy the ultrastructural features of the cardiac cells before and after the cold ischemia.

Group II - c-AMP variations

In a group of 11 patients (4 females and 7 males) with an average age of 51.3 years (range 27 to 71), operated on for six CABG, 2 closures of atrial septal defect (ASD) and 3 aortic VR with the same surgical technique we studied the concentrations of cyclic-adenosyl mono-phosphate (c-AMP) in cardiac biopsies of the right atrial appendage.

The average cross clamping time was 47.5 minutes (range 21 to 90). The tissue determination of c-AMP was performed by radio-immunological assay. The results are given in picomoles/mg of fresh myocardial tissue.

Group III: Anaerobic catabolism and beta-adrenoreceptor concentration

In correlation with a study of the blood gases and lactate, we studied the variations in the density of beta-adrenergic receptors before and after cold ischemic cardiac arrest in a series of 16 operated patients. There are 13 males and three females with an average age of 60 ± 2.7 years (range from 42 to 80). Twelve underwent CABG, 3 VR and one a closure of ASD. None of these patients had pre-operative beta-blockade drugs. No patient suffered from acute myocardial failure and none had been treated with catecholamines at least three weeks before surgery. The mean time of aortic cross clamping was 51.6 minutes.

A simple and fairly rapid method permits an assay of total receptor (beta 1 and beta 2) concentration using a specific radiolabeled ligand, i.e. 125 iodocyanopindolol. This method provides a direct measure of receptors density (B max) and affinity constant (Kd) in myocardial cells. The density of the beta-adrenoreceptors in atrial membrane preparation was determined by binding with the selective beta-adrenoreceptors radioligand (-) 125I-ICYP at concentrations ranging from 10-150 pmol/l.

The experimental data given in the text are means $\pm$ sem. The equilibrium dissociation constants (Kd) and the maximal number of binding sites (B max) were calculated from plots according to the method of Scatchard (6).

Group IV: Histo-chemical approach of the Kinetic of the catecholamines by Formaldehyde Induced Fluorescence technique. (FIF) (7)

In 20 patients, a right atrial biopsy was realized just before aortic clamping and after cold cardioplegic ischemia. In 13 of them, an associated left ventricular biopsy was made.

There were two females and 18 males with an average age of 65 ± 10 years. Cardiac procedures consisted of 10 CABG, seven aortic VR, one mitral VR and two closures of ASD in adults patients. The average of the aortic cross clamping time is 45 minutes.

The histochemical study of motor adrenergic innervation and catecholamine distribution in the cardiac tissue was founded on the fluorescence method of Falk and Hillarp.

Specimens obtained from unfixed cardiac biopsies were immediately frozen with carbon dioxide. Section cuts using a Reichert friocut cryostat were mounted on slides (35 microns), dried with silicogel and colored with Evans blue (50mg/l)(8).

After washing with water, the slide is dried and exposed to vapor from paraformaldehyde at 80°C (Falk 1962) with SO₄H₂ and water.

Observations were made using a Leitz Orthoplan microscope with a UV lamp Osram HBO. (Excitation filter BG 12 VG 1 and BG 3 VG 3. Barrier filter K 470 microns and 490 microns). Photographic images were obtained on Agfapan 25 films using a Leitz vario-orthomat system. The endogenous catecholamines in humans are Dopamine, Noradrenaline (NAD) and adrenaline (AD).

In three cases, a complementary histochemical study was realized with the osmium tetroxide-kalium iodine reaction. The biopsies are fixed in Karnovsky solution for 3 hours, rinsed in 0.1 mol/l phosphate buffer and post fixed in the OsO₄-KI mixture for 24 hours (9).

We have ethical approval and informed consent of the patients. In all these groups, the quantitative results were analyzed by the Student T test. A difference was considered statistically significant when $p < 0.05$.

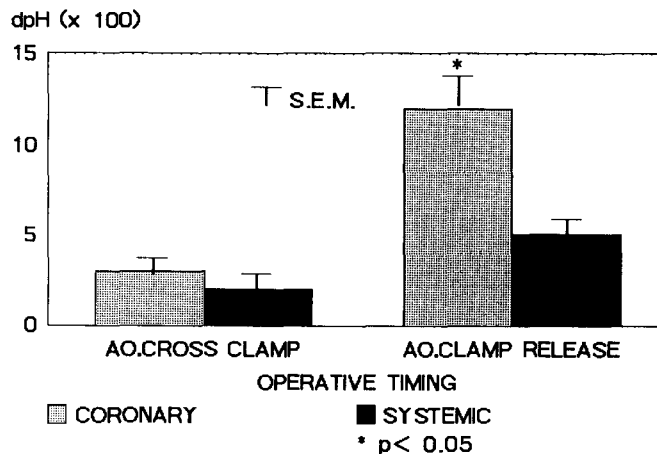
Results

Evaluation of the Anaerobic Changes During the Cold Cardiac Arrest:

Study of Group I: No significant differences of average age and average aortic cross clamping time between the two populations of patients with valve and coronary surgery.

The arterio-venous differences of pH between systemic venous level and coronary sinus is significant with a tendency to acidosis after clamping and release of the clamp (Figure 1).

FIGURE 1



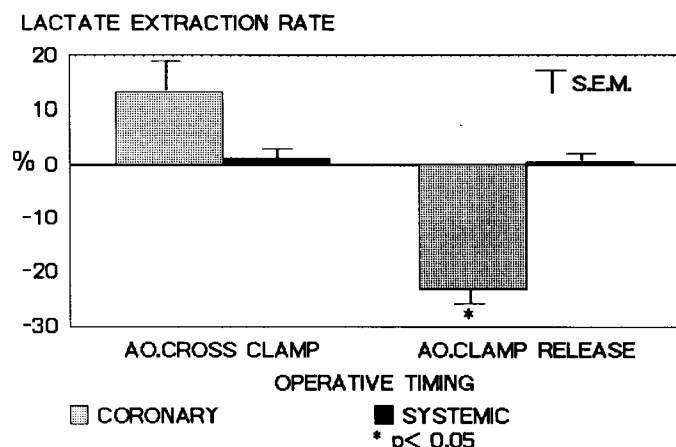
The average level of lactate extraction rate in overall patients of the Group I showed in relation to systemic blood a significant coronary excretion immediately after aortic cross clamping and after the ischemia during the release of the clamp (Figure 2).

There is no significant difference between the patients operated on for VR or CABG.

After this metabolic approach, we can ascertain that there is an ischemic myocardial injury with anaerobic coronary lactate excretion and decreasing of the coronary sinus pH level.

The anatomic features of the ischemia after electron microscopic study showed reversible damage in the myocardial cell.

FIGURE 2



We observe constantly two main modifications (Figure 3a and 3b):

- a mitochondrial swelling, and
- a transient decrease in the glycogenic cytoplasmic storage.

These two characteristics are shown immediately after aortic declamping and are progressively reduced after mechanical assistance and rewarming of the beating heart.

Are these morphologic and anaerobic metabolic changes deleterious for complex molecular structures as c-AMP, beta-adrenoreceptors and autonomic nervous neuro-transmitters?

Study of the Myocardial c-AMP During Cold Ischemia —

Figure 4 showed the average rates of c-AMP before and after aortic cross clamping in 11 studied cases.

We cannot observe significant differences between the average level of c-AMP before ischemia (1.173 ± 0.133) and after declamping (1.338 ± 0.222 pc mole/mg of fresh tissue weight).

The study of the correlation between the variations of concentration of c-AMP (Y) (post clamping level - pre-

FIGURE 3A

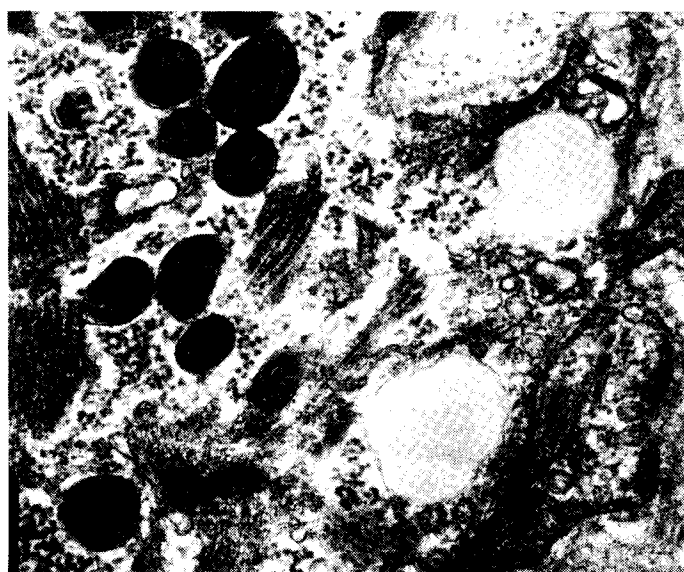


FIGURE 3B

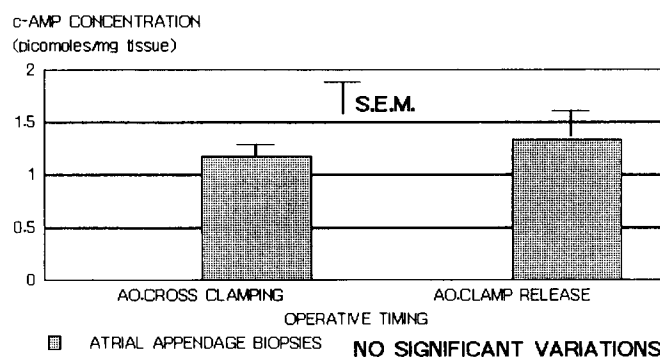


clamping level= dc-AMP) and the aortic cross clamping time (X) i.e. the cold ischemic time showed a significant relationship with 11 couples of measures: $Y = 1/(-22.7341 + 952.3024/X)$ with a coefficient of correlation of 0.624 (degree of liberty=9), $p > 0.05$.

The longer the ischemic time, the higher the increase of c-AMP concentration in the myocardial tissue.

FIGURE 4

VARIATIONS OF CARDIAC c-AMP CONCENTRATION AFTER COLD CARDIAC ARREST.



Study of the Myocardial Beta-Adrenoreceptors —

The study of the anerobic injury of the cold cardiac arrest with cardioplegia showed in this group a significant variation of the coronary sinus blood pH and a negative extraction rate of lactate. Consecutively, the excretion rate of plasma bicarbonate observed before the aortic clamping is inverted with a significant extraction rate to correct the metabolic acidosis (from $-122 \pm 66\%$ to $10.2 \pm 2.5\%$).

The measure of the density of myocardial beta-adrenoreceptors (B max) and the dissociation constant (Kd) showed that there is no significant variations in spite of the

anaerobic injury due to the cold ischaemia (Figures 5 and 6).

FIGURE 5

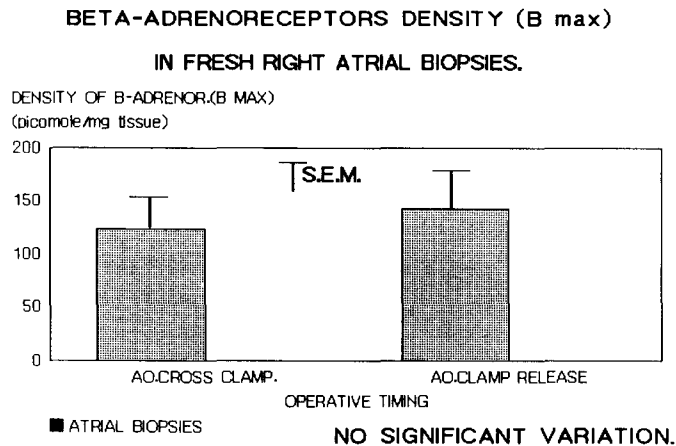
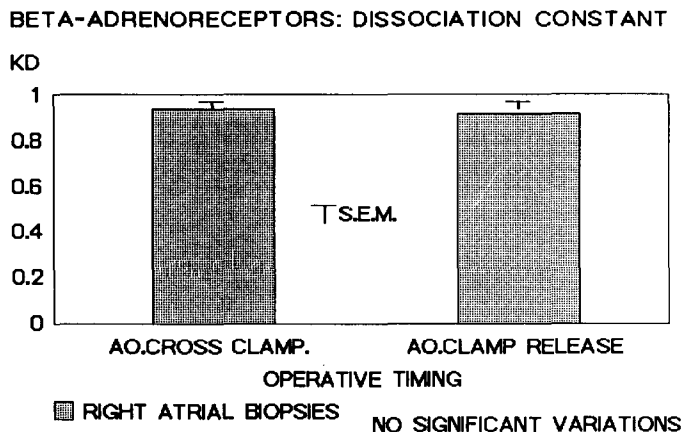


FIGURE 6



Histo-Chemical Morphologic Study of the Cardiac Catecholamines

The microscopic approach of the intracellular modifications of the kinetic of the catecholamines showed a redistribution between their different species.

Before ischaemia the FIF induced a high level of a yellow and gold fluorescence in the perinuclear area of the myocardial fiber cells. The length wave of the UV induced fluorescence is considered specific for histochemical identification of dopamine and serotonin (5-Hydroxytryptamine 5-HT).

We observe as well at the ventricular than at the atrial level the green intense induced fluorescence of the NAD and AD in catecholamine containing cell clusters. They may be interpreted as axonic cytoplasm of noradrenergic fibers in strength relationship with coronary endothelial cells and sub epicardial adipocytes. In this step of the operation the fiber content of NE appears strictly localized in multiple swellings of the axona (Figure 7a).

OsO4-KI reaction showed similar highly positive black granulations in noradrenergic nerve fibers (Figure 8).

After the ischemia, the histochemical geography of the different types of catecholamine seems to be redistributed:the

FIGURE 7A

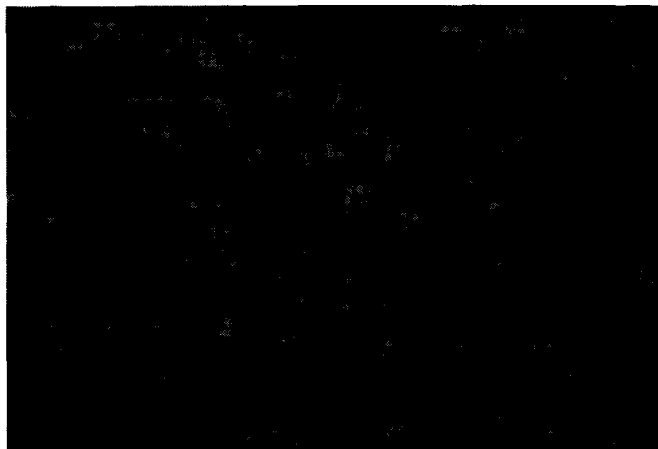


FIGURE 7A



FIGURE 8



intense perinuclear fluorescence of dopamine and serotonin disappears or is reduced and the initially intra-axonic NAD is released in the inter cellular space with diffuse green induced fluorescence (Figure 7b).

The ischaemia induced a sympathetic-like stimulation with release of an noradrenergic neurotransmitter previously contained in the fiber in the interstitial spaces and coronary capillaries. The decrease of the perinuclear content of dopamine

and 5-HT may be used to restore the NAD axonic storage in association to catecholamine uptake explaining the catecholamine turnover.

Discussion

This multiparametric study of the metabolic injury due to cold cardiac arrest intends to approach a better understanding of the pathophysiology of the ischaemia with protective cardioplegia in human heart during surgery.

The Anaerobic Aggression of the Cold Cardiac Arrest in Spite of the Cardioplegia

The normal heart had an almost exclusively aerobic metabolism extracting and metabolizing lactate and requiring a constant input in high energy phosphates (1, 2, 4). The main location of the cardiac metabolism is the mitochondria. The ischemia induced an anaerobic change with lactate excretion and acidosis. The production of lactate by the heart is the best indicator of myocardial ischemia (2, 10).

As witnesses of a degree of ischemia, in spite of the cardioplegia which induces a decrease of the oxygen consumption and stops the electrical cellular activity by the hypothermia and potassium, we can ascertain a significant acidosis with lactate extraction in the coronary sinus blood.

The mitochondria are very numerous in the myocardium composing between 25-50% of the entire myocardial mass (1). They are the main site of oxidative phosphorylation producing ATP molecules with energy contained in carbohydrates, lipids and proteins (11, 12). Their great number in the myocardial cell is commensurate with the important need of energy of the contracting heart. The heart maintains an almost constant content of glycogen (0.4 to 0.6% of fresh tissue weight) in the form of granules in the sarcoplasm (see Figure 3a) (13, 14).

Wollenberger et al (10) showed that few minutes after experimental hypoxia the intracellular glycogenolysis increased with anaerobic glycolysis and depletion in myocardial glycogen content. We also observed under cold cardioplegia a reduction of the intra cytoplasmic glycogenic storage associated with transient swelling of the ischaemic suffering mitochondria. (12-15).

c-AMP or the "SECOND MESSENGER"

It is now generally accepted that cardiac effects evoked by beta-adrenoreceptor stimulation are mediated by an increase in the activity of the adenylate cyclase/ cyclic AMP system (16). The stimulation of the beta-adrenoreceptors produces a rise in c-AMP levels in human left and right atrium and ventricles. Both subtypes (beta 1 and 2) are involved and contribute to adenylate cyclase activation (17). The relative contribution of each subtype remains controversial. The changes in adenylate activity and c-AMP levels are thought to activate inotropic and chronotropic response by elevation of intracellular Ca^{++} concentration.

The increase of c-AMP activates cAMP dependent protein kinase which in turn phosphorylates a component of the calcium slow or L channel and increases the calcium conductance and

myocyte calcium concentration.

The ischemia is a factor inducing the rise of intra cardiac cyclic nucleotides. Wollenberger et al. (10) found that a sudden rise of c-AMP occurred in the ischaemic heart. The accumulation of cyclic AMP in ischaemic tissue was superimposed on a high tissue lactate and tissue acidosis (18,19). This concept was supported by Dobson and Mayer (11). Podzuweit et al (18) found a relation between the rise of c-AMP and induction of heart fibrillation.

In our investigations the relatively stable c-AMP concentration in right atrial appendage tissues before and after aortic cross clamping showed the protective effect of the cardioplegia on energetic metabolic pathways in the cardiomyocytes.

The Beta-Adrenoreceptors

The autoradiography permits the localization of the B1 and B2 adrenoreceptors. In man, the density of beta-adrenoreceptors on coronary vessels is lower than that observed on the surrounding myocardium (17, 20).

After experimental induced ischemia in animals, cardiac membrane adrenoreceptors number is increased.

The physiological catecholamines NAD and AD produce a wide range of effects by activating multiple adrenoreceptors.

In the beta-subtypes, the inotropic, chronotropic and lipolytic effects were mediated by beta 1 receptors while vasodilator effects were produced by activation of the beta 2 receptors (17).

The two subtypes of beta adrenoreceptors are present in human atrial appendage as demonstrated by pharmacological studies of agonists and antagonists.

A presence of receptors reserve in the atria explain the maximal physiological response of the agonists by occupying only a fraction of the total receptors. There is less receptor reserve in the ventricle (21).

The Myocardial Distribution of Endogenous Catecholamines

Intrinsic cardiac innervation has been selectively studied with specific neurohistochemical methods in various species, including man. The adrenergic system has been mapped using FIF method (7). These studies have given confirmatory morphological evidence of the existence of adrenergic innervation in atrial and ventricular myocardium, in the sino-atrial and atrio-ventricular nodes, conduction system, and around blood vessels (22). Cells containing catecholamines have been observed in the atrial wall (See Figures 7 a and b, Figure 8) (3). A fairly rich distribution of brightly green fluorescing single varicose axons and small axon fascicles was observed by Waris et al (23) as in our biopsies before aortic cross clamping and cold ischemia. Dopamine appears to be stored in perinuclear area in myocardial cells and perivascular endothelial cells.

We observe after cold cardiac arrest an extra axonal diffusion of brightly green fluorescence of NAD and reduction of the dopamine store. It appears that terminal varicose plexus is responsible for noradrenaline liberation outside of motors

adrenergic nerve fiber (See Figure 7b).

Schomig et al (24) studied the contribution of centrally originating sympathetic activity to the myocardial extracellular accumulation of noradrenaline during the early phase of ischemia; they demonstrated the failure of a noradrenaline accumulation within extracellular space due to functioning neuronal uptake of noradrenaline and a failure of neurotransmission. For these authors, local factors seemed to play an increasingly important role in releasing NAD during the course of ischemia (25).

Conclusion

We intended a multiparametric approach of the metabolic changes in relationship to the cold cardiac arrest under crystalloid cardioplegia. The cardiac lactate excretion rate and acidosis in the coronary sinus blood permits to ascertain the significant degree of ischaemia with mitochondrial ultrastructural modifications.

This patent cold ischaemia did not appear to interfere on the beta-adrenoreceptors density and on the "second messenger" c-AMP concentration. The observation of an extra axonal release of NAD and depletion of the dopamine cellular store may be related to ischaemic sympathetic stimulation with failure of the uptake mechanism favoured by local factors as icosanoid liberation.

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