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Review

The past, the present and the future of experimental research on myocardial ischemia and protection

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Abstract:
At present, cardiovascular diseases represent the most important health risks because they are responsible for more than 50% of total mortality. Among them, ischemic heart disease is the leading cause of morbidity and mortality, and according to the World Health Organization, will be the major global cause of death by the year 2020. Major progress in the prognosis, diagnosis and therapy of ischemic heart disease would be impossible without notable achievements of the 20th century that have been critical for further development of cardiology. We are now living in the era of molecular medicine, and the influence of basic research on clinical practice has never been more pronounced. This, however, necessitates a new strategy; future cardiovascular research should include the following general guidelines: 1) to evaluate the role and proportion of already described molecular pathways; descriptive approaches will gradually disappear; 2) to distinguish between acute, chronic and pleiotropic effects of different drugs under *in vitro* and *in vivo* conditions, with respect to possible clinical use; 3) to use clinically relevant genetic models; 4) to study possible alterations in intracellular signaling in order to find the decisive steps responsible for abnormal control of cell growth, contractile function, lipid metabolism, cardiac ischemic tolerance, etc.; 5) to study the molecular mechanisms of cardiovascular diseases not only in healthy individuals, but also under different pathological conditions.
Such an approach must include developmental and gender differences, which are particularly important for the field of ischemic heart disease; therefore, experimental cardiovascular research can no longer be restricted to males of uncertain age. It is hoped that patients in future decades will profit from the progress of basic cardiovascular research.

Key words:
myocardial ischemia, protection, cell death, reperfusion

General background

The great Hippocrates was, unfortunately, mistaken when he stated that "The heart cannot become ill as then it would stop beating". We are actually facing a completely opposite situation: at present, cardiovascular diseases represent the most important health risk factors because they are responsible for more than 50% of total mortality. Among them, ischemic heart disease is the leading cause of morbidity and mortality, and according to the World Health Organization, will be the major global cause of death by the year 2020 [39]. Although the cardiovascular health status of our population has improved substantially and cardiovascular mortality has declined in recent years, we are still far behind the ideal situation.

The history of ischemic heart disease is relatively brief, showing the rapid development of cardiology as a scientific discipline [7, 46, 66]. Myocardial infarction was first described clinically in 1910, but the precise diagnosis was only possible after the introduction of the electrocardiogram into clinical practice in the 1920s. Before 1961, patients with acute myocardial infarction who were fortunate enough to reach the hospital were treated largely by benign neglect. They were sedated and placed on bed rest for five to six weeks. In 1961, Julian [27] articulated the concept of a coronary care unit, which includes the treatment of arrhythmias, cardiopulmonary resuscitation with external ventricular defibrillation and well-trained nurses. The introduction of the coronary care units caused an immediate 50% reduction in in-hospital mortality. Since 1963, in-hospital mortality has decreased stepwise by almost 70% with the introduction of thrombolysis, acetylsalicylic acid, invasive cardiology and cardiac surgery. Modern therapy, together with effective secondary prevention, has increased the two-year survival of patients after myocardial infarction by 75% over the past 30 years [66].

The progress in the prognosis, diagnosis and therapy of ischemic heart disease would have been impossible without several notable achievements of the 20th century, which were critical for further progress in the field of cardiology [37], e.g., the electrocardiogram, the Framingham Heart Study, the lipid hypothesis of atherosclerosis, coronary care units, echocardiography, thrombolytic therapy, heart catheterization and percutaneous coronary intervention, open-heart surgery and implantable defibrillators. It should be noted that the described achievements are the results of very close collaborations between theoretical and clinical cardiologists, and in almost every instance, these advances came from interdisciplinary and international collaborations [7]. This suggests that cardiology belongs to medical disciplines in which the cooperation of basic and clinical cardiologists has a long-lasting tradition of acting as an engine, driving scientific progress forward.

Although the management of ischemic heart disease centers on the development of effective primary prevention, the impact of these strategies may be limited. There is, therefore, an urgent need for effective forms of secondary prevention and, in particular, treatment that will limit the extent of evolving myocardial infarction during the acute phase of coronary occlusion. Based on these presumptions, cardiovascular research should concentrate on three consecutive periods during the development of myocardial injury: mechanisms involved in cardiac protection against ischemia, factors responsible for myocardial cell death and positive and negative consequences of myocardial reperfusion. Preserving the viability of ischemic myocardium should be the major therapeutic target.

Cardiac protection

The present

The degree of ischemic injury depends not only on the intensity and duration of the ischemic stimulus, but also on the level of cardiac tolerance to O_2 deprivation and other components of ischemia. Therefore, it is not surprising that the interest of many experimental and clinical cardiologists during the past 50 years has been focused on the question of how cardiac tolerance to ischemia might be increased.

In the late 1950s, the first observations appeared showing that the incidence of myocardial infarction was lower in people living at high altitude [26]. These epidemiological observations were later repeatedly confirmed in experimental studies using simulated hypoxia (for review see [48, 51]). In the early 1970s, interest concentrated on the possibility of limiting infarct size pharmacologically [36]; however, this effort was not successful because it became increasingly obvious that clinical observations did not correspond to the optimism of experimental results. After the period of skepticism, the discovery of a short-term adaptation of the myocardium, so-called "ischemic preconditioning", by Murry et al. [40] opened the door to the new era of cardiac protection. Several years after the description of acute cardiac protection by ischemic preconditioning (IP), a second delayed window of protection was observed [35]. At present, the long-term adaptation to chronic hypoxia (CH) (for review see [48, 51]) and short-term IP (for review see [71]) are examples of the potent cardioprotective phenomena. Both restrict infarct size, improve postischemic contractile dysfunction and reduce arrhythmias. The intensity of protection is stronger in IP, but on the other hand, the duration of protection is significantly longer after adaptation to CH (hours versus weeks, respectively) (Fig. 1).

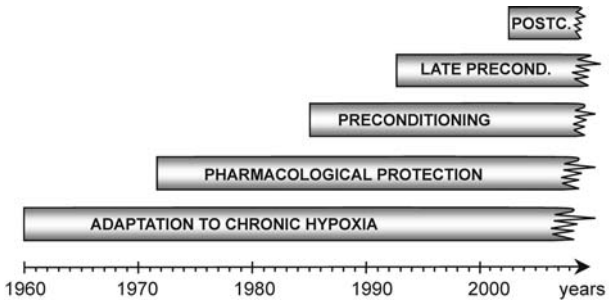

Fig. 1. History of cardiac protection

Unfortunately, the molecular mechanisms of cardiac protection have not yet been satisfactorily explained. Signaling for IP involves triggers (e.g., adenosine, several G-protein coupled cell-surface receptors and second messengers) and mediators (e.g., different protein kinases, free radicals, and NO), ultimately resulting in the activation of ATP dependent potassium channels (K_{ATP}) at the sarcolemma and in the mitochondria (for review see [4, 24, 38]). K_{ATP} expressed at the sarcolemma opens during hypoxia, ischemia or metabolic inhibition, thereby facilitating increased potassium influx and shortening of action potential duration. At the level of mitochondria, potassium flux across the inner mitochondrial membrane influences mitochondrial membrane potential, volume regulation, energy production and calcium homeostasis. Whether K_{ATP} channels merely play a signal transduction role or whether they are the end effector still remains to be established. Although a number of other end effectors have been proposed, such as the permeability transition pore and sodium/hydrogen exchanger, there are insufficient data available to support any one to the exclusion of the others.

Molecular mechanisms of the cardioprotective effect of adaptation to CH have been much less studied and the understanding of its signaling is still very limited (for review see [30]). Nevertheless it seems that various protective phenomena, including both short-lived IP and long-lasting effects of CH, utilize essentially the same endogenous pool of protective pathways, even if with different efficiency [42, 44]. In contrast to classical IP, CH not only activates these signaling pathways but also affects the expression of other proteins associated with maintaining oxygen homeostasis *via* transcription factors such as, e.g., hypoxia-inducible factor 1 (HIF-1α, for review see [64]). It is well known that exposure to chronic intermittent hypoxia is initially associated with oxidative stress [25, 28, 43] and increased adrenergic stimulation [49]. Both events were traditionally considered injurious but now it appears that they are also involved in the development of a cardiac ischemia-resistant phenotype. It has been proposed that the increased generation of NO plays a positive role in the protective mechanism induced by chronic hypoxia [2, 54]. Both adrenergic stimulation and increased production of ROS and NO can change the activity and/or expression of numerous signaling and effector molecules. Among them, various protein kinases have been studied regarding their role in the protection of chronically hypoxic hearts [41, 58]. Activated protein kinases may exert their protective effects by phosphorylation of numerous target proteins, such as the ATP-sensitive K^+ channel. A degree of controversy exists regarding the importance of the channel type that is localized either on the sarcolemma (sK_{ATP}) or the mitochondrial inner membrane (mK_{ATP}) [1, 29, 44, 54, 73]. Recent reports suggested the role of other factors, like maintaining intracellular calcium homeostasis [9], delayed mitochondrial transition [74], erythropoietin [8], and angiotensin II type 1 receptors [59]. Better understanding of the cardioprotective effect of CH and its possible application should be the subject of future research.

In this context it should be mentioned that there are two physiological situations where the heart is significantly more tolerant to ischemia: the immature heart and the female heart prior to menopause. Riva and Hearse [62] have observed that the ontogenetic development of cardiac resistance to global ischemia shows a biphasic pattern in rats, with increasing tolerance from the end of the first postnatal week up to the weaning period, followed by a decline through adulthood. The significant decrease of tolerance from day 1 to 7 [55] suggests a possible triphasic pattern of the ontogenetic development of cardiac sensitivity to ischemia, at least in rats (Fig. 2). Mechanisms underlying the higher resistance of the immature heart include increased anaerobic glycolytic capacity, better equipment for ATP synthesis, decreased free fatty acid uptake, and decreased sensitivity to acidosis as well as to calcium overload. Nevertheless, the mechanisms of the higher resistance have not yet been satisfactorily clarified and need further investigation. Thus the question arises of whether we can further increase the already high tolerance of the immature heart. It has been shown [55] that classical IP in rats is not

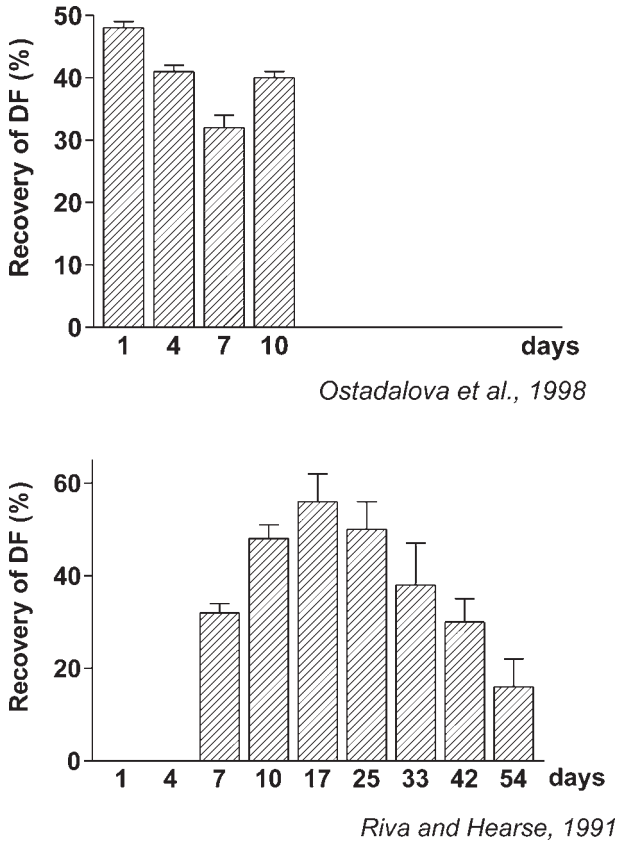

Fig. 2. Ontogenetic development of cardiac tolerance to ischemia

present at birth and that the enhanced postischemic recovery of contractile function only develops at the end of the first postnatal week. The decreasing tolerance of the neonatal heart to ischemia is thus counteracted by the development of endogenous protection. The same is valid also for the cardioprotective effect of adaptation to CH: no effect in neonates, the first signs of protection can be found during the first postnatal week.

Epidemiological studies have clearly shown that in women before menopause, ischemic heart disease originates about 10 years later than in men; the incidence of myocardial infarction is delayed by almost 20 years [14]. Most of the experimental studies have confirmed these results (e.g., [3, 33, 45, 52]). The molecular and cellular mechanisms responsible for this difference are, however, not yet fully resolved. It seems, however, that they are not only the result of the effect of sexual hormones on gene expression or activity of signaling cascades but that chromosomal differences have to be taken into consideration as well.

Clinical application

We agree with the statement of Bolli [4, 5], that myocardial protection is at crossroads. Over the past 40 years, hundreds of experimental interventions (both pharmacological and non pharmacological) have been reported to protect the ischemic myocardium in experimental animals. However, with the exception of early reperfusion, none has been translated into clinical practice, although a limited number appear to be quite promising in initial clinical studies. The barriers are both at the experimental and at the clinical level [5, 13]. The majority of experimental models do not adequately approximate actual clinical circumstances (e.g., isolated hearts, isolated myocytes and insufficient duration of ischemia). Conscious animal models (which might be most relevant to the clinical situation) have virtually disappeared; moreover, most of the experimental studies utilize healthy animals. The majority of basic research studies focus on molecular and cellular mechanisms of injury and protection rather than establishing the potential clinical efficacy of the interventions tested. Ischemic preconditioning remains disappointing due to several limitations including how to predict the onset of ischemia, short-lasting protection and the absence of appropriate pharmacological protection.

On the other hand, many of the clinical trials performed to date have been premature and not rationally designed. In addition, the multiplicity of factors that affect the patient's clinical conditions implies that large sample sizes are necessary to demonstrate that a change in any individual factor will change the outcome. In the clinical setting, the methods to measure infarct size (e.g., SPECT, enzymes) lack precision; the novel imaging techniques, such as delayed contrast-enhanced MRI are promising. Furthermore, our ability to monitor, in real time, active ischemia in patients is extremely limited: the appropriate biosensors are lacking.

As far as the clinical relevance of adaptation to CH is concerned, it is necessary to stress that CH can be found in common cardiopulmonary diseases, i.e., chronic ischemic heart disease and chronic obstructive lung disease. Clinical profit from this phenomenon depends upon the balance between the benefit (cardiac protection) and potential risks (pulmonary hypertension, right ventricular hypertrophy) [48]. Introduction of CH has, however, serious limitations re-

sulting from the complicated clinical accessibility of the simulated hypoxic environment.

Future directions

Perhaps the most pressing task for future research is to find out the end effectors of IP, including better characterization of the mitoK$_{ATP}$ channel, definition of its molecular identity and its direct physical or indirect relation to the mitochondrial permeability transition pore. Since most of the signaling pathways of IP (e.g., protein kinases, mitoK$_{ATP}$ channels and free radicals) are involved also in the mechanisms of adaptation to CH, it seems that they belong to general endogenous protective pathways [71]. This suggests that future research should involve both the redundancy and specificity of signaling. Future research should concentrate on the possible prolongation of the cardioprotective effect, which strongly limits the use of ischemic preconditioning in a clinical situation. Promising in this respect is the late phase of preconditioning: it has a broader range of protection and its duration is 30–40 times greater than that of the early phase; it is likely that it also has greater clinical relevance [5, 63]. Recent results indicate that cardiac transfer of the genes that mediate late IP (i.e., iNOS, COX-2, ecSOD) confers protection that emulates late IP [4]. The exploitation of both late IP and adaptation to CH thus should lead to the development of a permanently protected cardiac phenotype (prophylactic protection). An interesting aspect with regard to human susceptibility to myocardial infarction may be, therefore, to analyze the gender and age differences in cardiac tolerance to ischemia [31]. A particularly rewarding aspect of basic research in the field of cardioprotection should be the possibility of immediate translation of new discoveries obtained from *in vitro* experiments into the clinical setting, particularly those of coronary bypass surgery and percutaneous coronary intervention [47, 60].

Myocardial cell death

The present

Reduced blood flow to the myocardium causes metabolic, functional and morphological changes. At the level of the myocyte, both dysfunction due to impaired excitation-contraction coupling, electrical instability, altered ionic homeostasis and a shift from aerobic to anaerobic metabolism, on the one hand, and irreversible myocyte loss, on the other, are believed to contribute to disease progression. Cardiomyocytes can undergo cell death by two different mechanisms: necrosis and apoptosis [34]. Increased interest in apoptosis research in cardiology (in contrast to necrosis) mainly stems from the hope that understanding the mechanism of apoptosis in cardiac myocytes may provide new strategies to prevent myocyte loss. A major determinant for the success of this novel approach is the degree to which apoptosis contributes to total myocyte loss and to what extent this loss of contractile mass can be prevented to reduce functional deterioration and mortality. However, only a few reports provide evidence of the potential of anti-apoptotic therapy to improve the outcome in cardiac disease.

During the past several years, another form of cell death, autophagic cell death, has drawn considerable attention [15]. Autophagy is an intracellular phenomenon in which a cell digests its own constituents. Interestingly, the incidence of autophagy was described in the myocardium long before its implications were known [11]. Autophagy has been attributed to a number of cardiac disorders, such as ischemia and cardiac hypertrophy; it enables the cardiac cell to remove the "biological wastes", such as defective mitochondria and lipofuscin, accumulated in lysosomes and thus maintain cellular homeostasis [19]; stimulation of autophagy may thus have a cardioprotective effect. However, unlike in apoptosis, in which families of cysteine proteases and a number of other regulatory proteins have been identified, the mechanism of autophagic cell death remains unclear.

Future directions

There are still several unresolved issues in apoptosis that need to be addressed by future research [22, 34]. At the molecular level it still remains uncertain which mechanisms initiate the apoptotic process in cardiac myocytes. Although several interventions (e.g., catecholamines, atrial natriuretic peptide, angiotensin II) were shown to induce apoptosis in cultured myocytes, their role in human disease must be established. It is of major interest to discern the apoptotic pathways that lead to DNA fragmentation and positive TUNEL staining in clinically relevant experimental models;

they include, e.g., mitochondria-dependent *vs.* receptor-mediated pathways, and pro-apoptotic *vs.* anti-apoptotic signaling pathway. Furthermore, there are increasing doubts that evidence for apoptosis solely based on TUNEL staining and even DNA laddering may not be sufficient to prove apoptotic cell death; morphological alterations of myocytes *in situ* will be required. Of great importance is to define the role of myocyte apoptosis and its extent in the progression of disease and thus the possibilities of an anti-apoptotic therapy for human disease.

Autophagy can be described as a unique mechanism of maintaining cellular homeostasis [19]. In this context, it is likely that autophagy may play a crucial role in maintaining healthy myocytes in the myocardium. Therefore, an enhanced understanding of regulatory points that connect the process of autophagy with other intracellular pathways may open new possibilities for cardiovascular therapy. It is thus expected that the coming years will see a quantum increase in the understanding of autophagy in the context of the myocardium.

More studies are required to provide evidence for the pathophysiological importance and clinical outcome in order to support current hopes that apoptosis and autophagy of myocytes will become a new target for future therapeutic intervention.

Reperfusion

The present

In the clinical treatment of evolving acute myocardial infarction, early coronary reperfusion has proved to be the only way to limit the infarct size. However, there is also evidence from animal studies that reperfusion may contribute to further tissue damage, a phenomenon known as "reperfusion injury"; it includes arrhythmias, enzyme release, or severe intramyocardial hemorrhage and cell death. Oxygen free radicals were shown to be generated upon restoration of blood flow and to be potentially harmful, and their role as the main mediators of reperfusion injury were soon widely accepted [16]. Piiper et al. [57] have suggested three initial causes of immediate reperfusion injury, apart from oxygen radicals: re-energization, rapid normalization of tissue pH and rapid normalization of tissue osmolality. Taken together, one can conclude that during ischemia-reperfusion the cardiomyocytes are exposed to a sequence of adaptive and injurious events.

However, the concerns about the potential clinical significance of reperfusion injury were soon assuaged by the results of clinical studies. The data obtained in millions of patients with an evolving acute myocardial infarction has demonstrated that reperfusion therapy may be more or less beneficial, depending on the circumstances, in particular on how early it is applied (e.g., Prague study [68, 69]). Reperfusion injury was, therefore, considered by many clinical cardiologists to be either non-existent (reperfusion associated phenomena as accelerated expression of pre-existent injury) or clinically irrelevant (in relation to the importance of ischemic injury) [18]. It must be mentioned that, in contrast to the skepticism of cardiologists, many cardiovascular surgeons are convinced of the existence of the potentially adverse effects associated with restoration of normal myocardial perfusion [60]. Both experimental and clinical cardiologists, however, agree that the main target in reperfusion is the restoration of microcirculation; the most striking example of postischemic microvascular incompetence is the so-called no-reflow phenomenon [53].

The concept of reperfusion injury has thus been a subject of debate for the past three decades, in which some investigators believe that all injury develops during the ischemic period, whereas others argue that blood reflow extends tissue injury. However, the discovery of postconditioning [67, 72] in recent years has bolstered the concept of reperfusion injury. The term postconditioning refers to the phenomenon in which multiple rapid interruptions of blood flow in the early phase of reperfusion result in a reduction of infarct size. This cardioprotective mechanism is more clinically relevant than that of IP: intermittent ischemia/reperfusion is applied after a prolonged coronary occlusion. The protective effect of postconditioning is comparable with that of IP and has been described in different animal species [17, 72]. Importantly, evidence for the existence of the protective effect of postconditioning has also been obtained in patients experiencing an acute myocardial infarction [32, 65]. It was observed that postconditioning involved signal transduction pathways similar to preconditioning (e.g., NOS and mK_{ATP} [70]).

In recent years studies have been devoted to the mitochondrial permeability transition pore (PTP); its

role in reperfusion injury was hypothesized at the end of the 1980s [10]. The PTP is a voltage-dependent, high-conductance channel located in the inner mitochondrial membrane. At present, it is widely accepted that PTP opening contributes to the loss of viability associated with post-ischemic reperfusion: indeed, conditions associated with post ischemic reperfusion, such as ROS accumulation, pH normalization and rise in calcium ions, create an ideal scenario for PTP opening (for review see [12]). Since PTP inhibition prevents reperfusion injury, it was hypothesized and proven that the powerful protection associated with postconditioning could be attributed to a decreased probability of PTP opening [21]. The concept that protective intervention can be used after the onset of ischemia is thus receiving increasing attention and might be exploited in clinical settings [56].

Future directions

Future research should, therefore, bring together the opinions of basic research and clinical cardiologists. From the experimental point of view it would be desirable to better understand the molecular mechanisms involved in the initial minutes of reflow, to determine whether the mechanisms originate in cardiomyocytes and/or in vascular or blood-borne cells and whether reperfusion induces death of non-injured cells or accelerates the death of cells, injured during the period of ischemia [20]. Of great importance would be the clarification of the role of apoptosis in ischemia/reperfusion injury [22] and the function of mitochondria in this process, particularly through the opening of the permeability transition pore [12, 18]. A new approach concerning the reperfusion injury salvage kinase (RISK) pathway, suggested by Hausenloy and Yellon [23], may prove interesting. Therapeutic relevance involves reduction of reperfusion injury and restoration of microcirculation based on treatments targeting the previously discussed mechanisms in order to minimize the impact of acute coronary syndromes on survival and the quality of life.

Conclusions and perspectives

We are living in an era in which the influence of molecular medicine and basic research on clinical practice has never been more pronounced. The recent achievements in molecular biology and genetics opened the door for substantial progress of many medical disciplines, including cardiology. This, however, requires a new strategy for future cardiovascular research. The huge amount of available genetic information should now be exploited for integrative cardiovascular pathophysiology. Such an approach must, first of all, include developmental and sex differences, which are particularly important for the field of ischemic heart disease; therefore, experimental cardiovascular research can no longer be restricted to males of uncertain age. Clinical epidemiological studies have shown that ischemic heart disease is no longer the disease of the fifth and higher decades of life, but its origin and consequences may be essentially influenced by risk factors that are already acting during early development. Moreover, it is necessary to mention that healthy, immature myocardium is more tolerant to ischemia than the myocardium of adults, and that the cardioprotective effects of adaptation to CH and IP are significantly less expressed during early ontogeny [54]. Therefore, it follows that experimental studies of the pathogenetic mechanisms of myocardial injury and protection must shift to the early ontogenetic period [50]. Sex differences have a pronounced influence on the type and severity of cardiovascular diseases, including the susceptibility to ischemic heart disease [31]. Experimental results indicate that some sex differences may exist even at the molecular level (e.g., a different number of K_{ATP} chan-

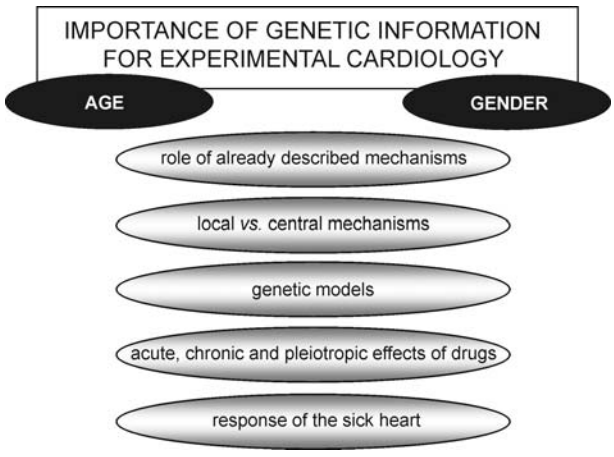

Fig. 3. Future cardiovascular research – general aspects

nels, and the microvascular generation of free radicals; [61]). There is increasing evidence that the field of cardiology is not exempt from the basic biological fact that men and women are different, and this may need to be reflected in the therapies they receive [6]. Basic research should follow this imperative.

Future cardiovascular research should include the following general aims [43] (Fig. 3):

1) evaluation of the role and proportion of the already described molecular pathways; the descriptive approaches will gradually disappear;
2) distinction between the acute, chronic and pleiotropic effects of different drugs under *in vitro* and *in vivo* conditions, with respect to possible clinical use;
3) use of clinically relevant genetic models;
4) study of possible alterations in intracellular signaling to find the decisive steps that are responsible for the abnormal control of cell growth, contractile function, lipid metabolism, cardiac ischemic tolerance, etc.; and
5) study of the molecular mechanisms of cardiovascular diseases not only in healthy individuals but also under different pathological conditions.

It is hoped that patients in future decades will profit from the progress of basic cardiovascular research allowing many useful treatments to become available. Simultaneously, there should be a greater focus on prevention, using different early markers of myocardial injury. Moreover, the development of suitable animal models of human cardiovascular diseases is very much needed and remains as important as ever.

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