The Role of G-Proteins in the Pathophysiology of the Cardiovascular Diseases

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Questions exist about the mechanisms by which cells of the cardiovascular and other specialized systems recognize and respond to hormones and neurotransmitters. The search for answers to these fundamental questions about the innermost workings of pathophysiological mechanisms has prompted a tremendous effort in research over the last 30 years and has led to new frontiers of knowledge, which have resulted in more than one Nobel Prize. During the last 20 years, the use of biochemical and molecular methods has revealed that the beginning of the cellular response occurs in membrane receptors. Several types and subtypes of these receptors have been identified, defining essential regulatory molecules such as acetylcholine and noradrenaline. For example, so far, five different cholinergic receptors and five different adrenergic receptors have been cloned and sequenced. All of these receptors are glycoproteins that have regions with substance, sequential identity, and seem to be part of a great family of receptors that share structural similarities. The occupation of the receptors by "primary messengers" such as the catecholamines and acetylcholine, regulate the activation of one or more systems of effector "secondary messengers" in responsive cells. These systems include ion channels and enzymes, such as adenylate cyclase (which forms cAMP) and guanylate cyclase (which forms cGMP) [1,2].

At the beginning of the 1970s, Rodbell et al speculated about the presence of more than one component in the activation of adenylate cyclase by hormones, after discovering the involvement in a stage where there was participation by guanosine triphosphate (GTP). The hydrolysis of GTP stimulated by hormones, observed by Cassel and Selinger, was significant. The discovery by Gilman et al of a membrane factor different from a receptor or from adenylate cyclase led to the identification of this element. In 1987, Alfred Gilman extensively described this component as a family of proteins called G-proteins, which bind the family of receptors and the intracellular effector molecules [1,2].

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This review aims to describe the innermost mechanisms of cardiovascular diseases at the biochemical level, emphasizing the role of G-proteins. The text begins with the presentation of basic concepts about the interaction between "messengers" that are part of the event cascade that leads to intracellular responses of specialized cells. At the subsequent stages, the properties of G-proteins are discussed as well as their role on the cAMP and cGMP vias, stressing their importance in specific cardiovascular diseases. This review does not intend to exhaust the subject, but to simplify it as much as possible, so that the readers of the *Arquivos Brasileiros de Cardiologia* can recycle their knowledge about the innermost mechanisms of cardiovascular diseases.

Basic Concepts

There are mechanisms by which a biochemical signal generated by a hormone or neurotransmitter causes a biological effect inside a cell. These mechanisms are generically designated as "signal transduction" and can be divided into two basic groups: 1) transduction through intracellular receptors; 2) transduction through cell surface or membrane receptors (table I) [3].

Intracellular receptors - In this system, the transduction of the biochemical signal is performed through intracellular receptors bound to the cytosol or to the nucleus. The complex receptor-agonist binds specific regions of the DNA, causing an increase in the expression of specific genes. In this case, the effects of the agonists are not immediate because time is needed for the genic transcription and for the subsequent translation of the mRNA. Some examples of this transduction through intracellular receptors are: steroid receptors, vitamin D and retinoic acid receptors, and thyroxin receptors [3].

Membrane receptors - In the transduction systems that use this type of receptor, the signal is transferred to the intracellular processes responsible for the cellular responses through a sophisticated system in which the G-proteins that act in the cAMP and cGMP systems take part. These proteins represent a family whose members include the following: 1)

Gs – stimulate the adenylate cyclase through beta-adrenergic receptors, and have a recently discovered effect of increasing the conductance of calcium in the heart; 2) Gi – seems to have multiple methods of inhibiting adenylate cyclase through the alfa2-adrenergic receptors; 3) Gt – also known as transducin, binds the photoreceptor rhodopsin in the retina; 4) Go – can regulate calcium channels; 5) Gk – regulates the potassium channels; 6) Gp – not yet well defined, can regulate phospholipases (table II) [3-6].

All G-proteins are structurally very similar, being composed of three subunits designated as alpha (α), beta (β), and gamma (γ). The alpha-subunit is the most characteristic of each G-protein. This is the subunit that usually interacts with the receptor, binds GTP, and regulates the effector systems. The G-proteins can be studied by the use of bacterial toxins, especially the cholera and pertussis toxin. The cholera toxin stimulates the Gs-proteins, and the pertussis toxin inactivates the Gi-proteins. The effects of these toxins have been tested in cellular cultures and in cellular membranes, and lymphocytes and monocytes of patients with heart disease have been used as substrates. However, all these methods are very limited for clinical use on a large scale [3-6].

The activation of the regulatory G-proteins is associated with the agonist stimulation of the majority of the receptors bound to the cellular membrane. Therefore some agonist-receptor interactions facilitate the GTP binding with an alpha-subunit of the protein and the G-protein is activated. Then, this G-protein dissociates from the receptor causing decrease of affinity between the receptor and the agonist, and the alpha-subunit is released (figure 1). The distinct alpha-subunits derived from the different G-proteins can activate several intracellular processes. The identity of this G-protein and its relation with the G-proteins involving the alpha1-adrenergic stimulation of phospholipases C are unknown [3-6].

Unlike the intracellular receptors, the membrane receptors do not directly regulate the genic expression and can have indirect effects. Based on signal transduction mechanism, there are four classes of membrane receptors: 1) neurotransmitters bound with ion channels of nerves and muscles (ex. nicotinic cholinergic receptors, GABA receptors, and receptors of glycine); 2) catalytic receptors related to the enzymatic activity as part of its structure, and in the majority of the cases the enzymatic activity is a tyrosine-specific protein kinase (ex. insulin receptors); 3) receptors related to intracellular secondary messengers, which act as real signal amplifiers, because one molecule of the receptor activates several molecules responsible for the intracellular effects. In this case, many ion channels are not directly coupled with their receptors, but work through G-protein action [3].

It should be stressed that the idea that G-proteins provide the only system of signal transduction through receptors is false. Cumulative efforts of several laboratories have contributed to the elucidation of signaling mechanisms independent from G-proteins. These mechanisms involve activation of several signaling membrane molecules, followed by a sequential stimulation of various protein kinases collectively known as MAPK (mitogen-activated protein kinase). The MAPK signaling cascade amplifies and transmits signals that, eventually, activate several regulatory molecules in the cytoplasm and in the nucleus to start cellular processes such as proliferation, differentiation, and development. This cascade is not only restricted to the signaling of growing factors. It is also related to pathways started by phorbol esters, ionophores, and heat shock proteins.

The hormones, the neurotransmitters, and the growth factors can be considered signals, and the receptors detectors of these signals. Each component functions as an element of communication between extracellular events and chemical alterations inside the cell. Molecules considered "secondary messengers" (so-called because they function as an element of binding or transduction of the signal originated in the receptors and the final cellular effect) are part of an event cascade that transforms the binding of a neurotransmitter or hormone in a cellular response. The two secondary messenger systems more widely studied are the adenylate cyclase system and the calcium/phosphatidylinositol system [3,4].

Cyclic AMP system (cAMP) - The adenylate cyclase is related to chemical signals emitted from the beta and alpha2 receptors, whose actions in intracellular processes are mediated by G-proteins. Many active G-protein molecules are formed from the activation of a single receptor. The ability of the hormone or the neurotransmitter to stimulate or inhibit the adenylate cyclase depends on the

Table I – Signal-transducing mechanisms

1)	Intracellular receptors: steroid receptors, vitamin D receptors, retinoic acid receptors, and thyroxin receptors.
2)	Membrane receptors directly coupled with effector molecules.
A)	Receptors bound to ion channels: cholinergic nicotinic GABA receptors.
B)	Receptors with catalytic activity: insulin receptors.
1)	Membrane receptors coupled with effector molecules through secondary messengers.
A)	Receptors coupled with adenylate cyclase: adrenergic receptors, glucagon, and epinephrine.
B)	Receptors coupled with the phosphatidylinositol cycle and diacylglycerol: muscarinic receptors, receptors A1, and growing factors.

Table II – Main G-protein families

1)	Gs - stimulates adenylate cyclase through beta-adrenergic receptors and increases calcium conductance in the heart.
2)	Gi - seems to have multiple forms of inhibiting adenylate cyclase through alpha2-adrenergic receptors.
3)	Gt - also known as transducin; binds the photoreceptor in the retina.
4)	Go - can regulate calcium channels.
5)	Gk - regulates potassium channels.
6)	Gp - not yet well defined, can regulate phospholipases.

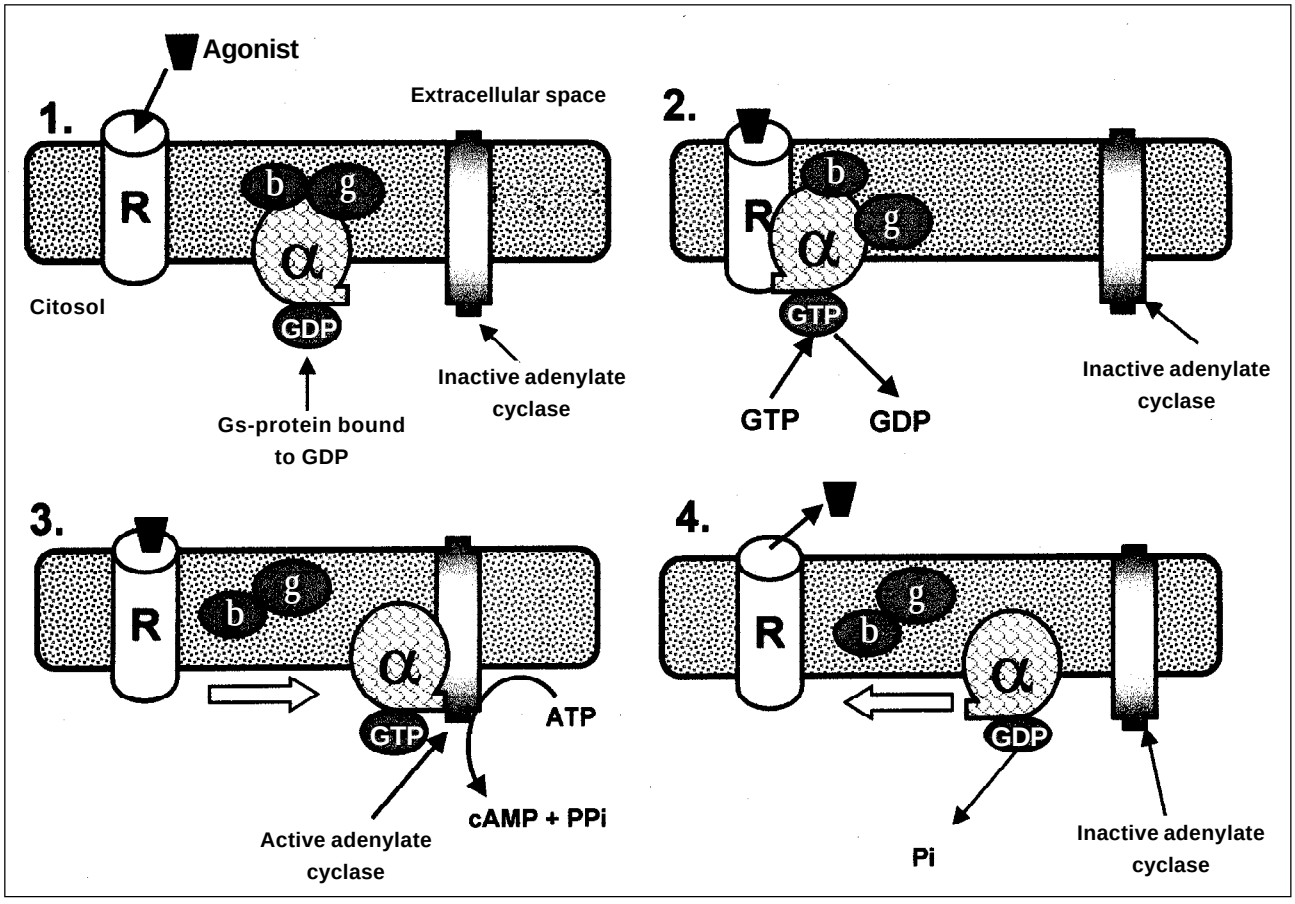

Fig. 1 – Recognition and signal transduction related to adenylate cyclase system by coupling between receptor and Gs-protein. 1. Rest condition: free receptors do not interact with Gs-proteins; 2. G-protein binding to the receptor activated by a hormone or neurotransmitter: the occupied receptor changes its shape and interacts with Gs-protein that releases GDP and binds to GTP; 3. Activation of adenylate cyclase: the alpha-subunit of Gs-protein dissociates and activates the guanylate cyclase; 4. Inactivation of adenylate cyclase: GTP is hydrolyzed in the alpha-subunit to GDP and adenylate cyclase is inactivated, returning to resting condition. (Adapted[3]).

G-protein type: stimulating (Gs-protein) or inhibiting (Gi-protein) (figures 2 and 3) [8-10].

The next step of the cAMP "secondary messenger" system is accomplished through a family of enzymes called cAMP-dependent protein kinases or protein kinases A (PKA), which by the transformation of ATP in ADP promotes the phosphorylation of the proteic substrate responsible for the intracellular effects. Not all protein kinases respond to cAMP. There are several types that are independent from this system, such as the protein kinase C (PKC) [3,4].

Many receptors respond to the action of hormones and neurotransmitters by activation of a membrane phosphodiesterase called phospholipase C, which is also activated by the signal transduction by G-proteins. The activation of the phospholipase C releases two fragments: 1,4,5 phosphatidylinositol triphosphate (IP3) from diphosphate (PIP2), and diacylglycerol (DAG). These two molecules have synergic actions with the secondary messenger molecules. IP3 binds to receptors of the endoplasmic reticulum, causing a rapid release of calcium from intracellular reserves, allowing the formation of the complex calcium-calmodulin, which is a mediator of several enzymatic actions. IP3 is a chemical signal of short life. It is rapidly dephosphorylated in PIP2 and PIP, which are inactive as secondary messengers. DAG seems to increase the PKC affinity for calcium. Both secondary messengers (IP3 and DAG) act synergically causing the protein phosphorylation necessary for the intracellular effects [3,4].

Cyclic GMP system – cAMP and calcium are the two most widespread secondary messengers. However, the cells have more specialized signaling systems, including the guanylate cyclase system (cGMP) and the nitric oxide (NO). cGMP is similar to the cAMP system in many ways, including the membrane receptors and G-proteins. Guanylate cyclase, however, differs from adenylate cyclase because it can be an integral part of the receptor, being, therefore, similar to the tyrosine-specific protein kinases. Many tissues contain a form of cytosolic guanylate cyclase, non-coupled with membrane receptors. Unlike cAMP, which affects a wide range of processes, cGMP functions as a specialized messenger, related to smooth muscle relaxation, platelet aggregation, and the visual system [3,4].

The cGMP system began to be exhaustively studied in regard to the pathophysiology of the cardiovascular diseases because it is the final effector system of the NO action and also because of the importance of the recognition of the endothelial dysfunction as a cause or consequence of

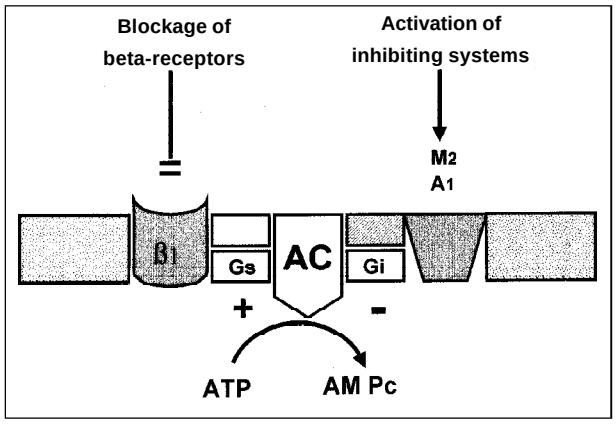

Fig. 2 - Simplified sketch of adenylate cyclase system including stimulating and inhibiting receptor systems. Adenylate cyclase (AC) is activated by beta1-adrenergic receptors (beta1-AC) via stimulating G-proteins (Gs) and inhibited by inhibiting receptor systems (M2 – M2 muscarinic receptors; A1 – A1 adenosine receptors) via inhibiting G-proteins (Gi). Adenylate cyclase activation can be prevented by beta1-blockers or, alternatively, by the activation of inhibiting receptor systems. (Adapted[10]).

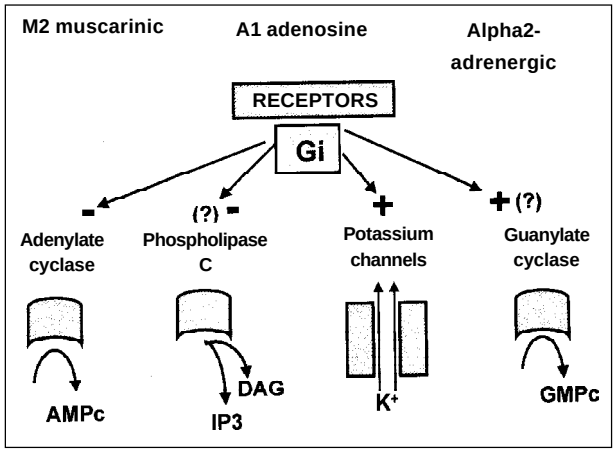

Fig. 3 - Inhibiting G-proteins and their relation with varied types of receptors and cellular responses. Gi - inhibiting G-protein; DAG - diacylglycerol; IP3 - inositol triphosphate.

cardiovascular diseases. The event cascade that participates in the production and release of NO is essentially similar to the event cascade of the cAMP system. There is also the participation of the G-proteins binding the information coupled with membrane receptors of the endothelial cell to the phosphatidylinositol pathway, which releases calcium from its endoplasmic reserves. After the agonist stimulation of the endothelial cell, via receptor/G-protein, the PIP2 phosphodiesterase acts on the phosphatidylinositol 4,5-diphosphate (PIP2) to generate diacylglycerol (DAG) and inositol-triphosphate (IP3). Then, IP3 mobilizes the intracellular calcium while the PKC activated by DAG promotes the influx of the extracellular calcium. This hypothesis is consistent with the finding that, in culture of endothelial cells, bradykinin or ADP stimulates phospholipase C to release IP3. In addition, the exogenous phospholipase C can cause endothelium-dependent relaxation, while the phospholipases B and D cannot. Furthermore, the endothelium-dependent vasodilatation is empowered by the inhibition of diacylglycerol kinase. The enzyme that starts the conversion of L-arginine to NO by the endothelium, called nitric-oxide synthetase, is activated by the increase of the cytosolic calcium, which is mediated by calmodulin.

G-proteins, endothelium, and nitric oxide - The release of EDRF/NO (endothelium-derived relaxing factor/nitric oxide) or other EDRFs, because it is postulated that there are more than one endothelial factor, can occur through different pathways involving G-proteins and independent mechanisms. The Gi-protein is responsible for the mediation of inhibitory effects of receptors in the adenylate cyclase pathway. Little is known about the signal transduction involving the synthesis or release of EDRF/NO. An early stage of the majority of the responses mediated by receptors is the activation of G-proteins in the cellular membrane, which can initiate the modulation of a variety of intracellular events (figure 4)[8].

The role of G-proteins in the pathophysiology of the vasospasm after global ischemia and reperfusion is also unknown. Their participation was documented through comparative study of vascular relaxation caused by sodium fluoride, which could produce biphasic responses in human, bovine, and porcine coronary arteries, causing specifically an endothelium-dependent relaxation and an endothelium-independent contraction. Fluoride seems to release an EDRF with characteristics similar to NO. It also seems to release a prostanoid that is sensitive to indomethacin and can be similar to the one described. Fluoride acts stimulating G-proteins to release these EDRFs. The dysfunction of G-proteins in the endothelium has also been postulated as responsible for the endothelial dysfunction in conditions of endothelial cell regeneration after injury, atherosclerosis, and coronary vasospasm (figure 5) [6,8].

G-proteins, cyclic AMP system, and cardiovascular diseases

Myocardial ischemia – Several alterations seem to be responsible for the loss of function of adenylate cyclase in the ischemic myocardium. There is a reversible phase of this process characterized by the uncoupling of the G-protein receptors and a supposed allosteric alteration of the catalytic subunit, causing an increase of calcium in a compartment next to the enzymatic activity. The irreversible alteration of the adenylate cyclase function, observed in ischemia that lasts more than 30 minutes (global normothermal ischemia), is assumed to be mainly, if not exclusively, caused by the action of free radicals.

Several studies have described an increase, instead of a decrease, in the density of beta-adrenergic receptors in the plasmatic membrane of ischemic hearts. Due to the loss of high-energy phosphates in acute myocardial ischemia, the coupling of beta receptors and signal transduction is completely halted, with a preponderance of external

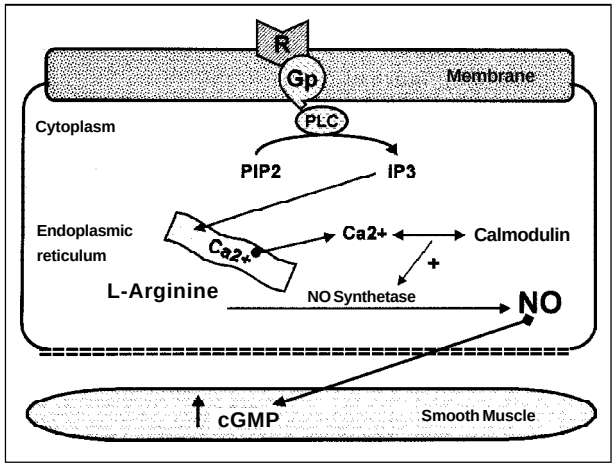

Fig. 4 - Nitric oxide release pathway. R - receptor; Gp - G-protein; PLC - phospholipase C; PIP2 - inositol diphosphate; IP3 - inositol triphosphate; NO - nitric oxide.

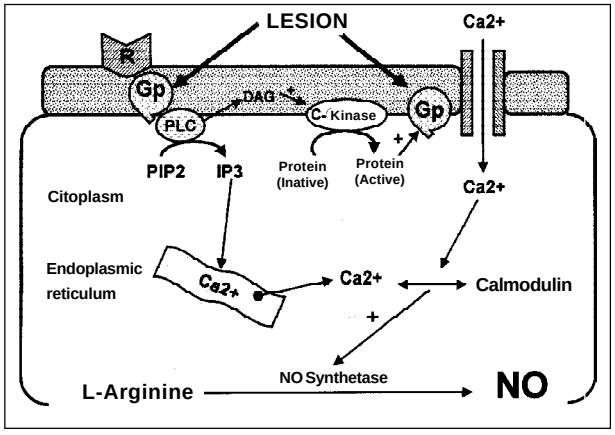

Fig. 5 - In the ischemia/reperfusion injury, there is an impairment of nitric oxide release due to a defect of signal transduction through the membrane. The endothelial cells maintain their capacity of nitric oxide production and release. This is a pattern of response to injury in all cardiovascular diseases. R - receptor; Gp - G-protein; PLC - phospholipase C; C-kinase - protein kinase C; PIP2 - inositol diphosphate; IP3 - inositol triphosphate; NO - nitric oxide. (Adapted from Evora [5,6]).

phenomena in relation to internal ones, leading to an increase of beta-receptors in the cellular surface. In the first pathophysiological step of myocardial ischemia, during the early desensitization, the uncoupling of G-protein receptors is completely halted. Even the exogenous administration of catecholamines to ischemic hearts cannot compensate for this effect [9,10].

As already mentioned, adenylate cyclase is regulated by stimulating and inhibiting vias. Beta-adrenergic receptors activate G-proteins increasing enzymatic activity, while the activation of the M2 muscarinic receptors and of the A1 adenosine receptors leads to activation of Gi-proteins, which reduce the enzymatic activity. This adenylate cyclase inhibition reduces both its basal and stimulated activities. Therefore, a tonic increase of the inhibiting activity of Gi-proteins results in a decrease of the response of adenylate cyclase to stimulating hormones. The loss of the tonic inhibition, on the other hand, leads to an increased responsiveness or sensitivity of the adenylate cyclase system. This functional imbalance of G-proteins has been observed in many models of acute myocardial ischemia. While Gs-protein levels remain intact during a long period after the onset of an ischemic injury, Gi-protein levels rapidly loose their functional activity. The molecular mechanism of this functional impairment needs further study. With the extension of ischemia, a general decrease in adenylate cyclase activity was detected in many experimental models of acute myocardial infarction (AMI). Recent studies detailing the evolution of the acute ischemia have shown a very rapid but transitory activity of adenylate cyclase independent from beta-adrenergic receptors and G-proteins. This activity can be associated with protein kinase activation [11].

One of the major complications of myocardial ischemia is the occurrence of malignant tachyarrhythmias, which can be partially explained by a transitory increase of cellular sensitivity to the stimulation of adenylate cyclase by catecholamines. Consequently, the prevention of the stimulation of this enzyme by beta-blocking agents is efficient in the reduction of the incidence of these arrhythmias. Therefore, the activation of guanylate cyclase can also be prevented by endogenous mechanisms through Gi-protein activation mediated by M3 muscarinic receptors, by A1 adenosine receptors, and perhaps by alpha2-adrenergic receptors. The activation of Gi-proteins in myocardial ischemia may have some advantages, because they not only represent an inhibition of the adenylate cyclase but also promote hyperpolarization in the atria and in the conducting system. According to these considerations, there is increasing evidence that the activation of the cholinergic system may prevent tachyarrhythmias induced by ischemia. The potential benefits of the cholinergic stimulus, however, can be attenuated because the inhibition of the pre-synaptic release of noradrenaline, mediated by muscarinic receptors, is reduced during ischemia. In addition, the response of the ischemic myocytes to cholinergic stimuli mediated by M2 muscarinic receptors is reversibly reduced during short periods of ischemia. This can lead to reduction of the adenylate cyclase inhibition and, therefore, contribute to the mechanisms of arrhythmia induced by ischemia. The prevention of the alterations induced by ischemia in signal transduction by Gi-proteins and/or selective activation of M2 muscarinic receptors could be, with some restrictions, a pharmacological alternative in the treatment of acute myocardial ischemia [10,11].

The occurrence of malignant arrhythmias and the extension of the infarcted area followed by heart failure (HF) determine the clinical evolution of the AMI. The activity of the adrenergic system plays a major role in this evolution because acute myocardial ischemia on several levels leads to an inadequate activation of the adrenergic system with a rapid increase of beta-adrenergic activity and functional impairment of Gi-proteins. In addition to these two mechanisms, the transitory sensitization of adenylate cyclase in the acute myocardial ischemia occurs due to a change in the enzyme, which can only be totally blocked by an inhibition of the protein kinase C. So, in addition to

functional alterations of receptors and Gi-proteins, the acute myocardial ischemia leads to a rapid activation of the protein kinase C by mechanisms not yet completely understood. This process can directly activate the ion channels and the change Na+/H+, and influence in the evolution of AMI.

To complete the specific review on myocardial ischemia, something about remodeling should be said. Large transmural infarctions lead to impairment of cardiac remodeling, increasing the risk of congestive heart failure (CHF). Angiotensin II, endothelin, and alpha1-agonist receptors are implicated in the development of myocardial hypertrophy, interstitial fibrosis, and HF following AMI. Due to the fact that these agonists are coupled and activate a Gq-alpha-protein in the heart, it is possible that this protein is involved in remodeling mechanisms and HF following AMI. Studies on experimental myocardial infarction in rats show an increase of Gq-alpha-protein concentration in the normal myocardium, in the border of the infarcted area, and in healing tissues after 8 weeks of evolution with moderate HF. An apparent increase in expression of phospholipase C was also observed, as well as a significant elevation of the activity of the phosphatidylinositol cycle in the borders of the infarct and healing tissues. These observations indicate that Gq-alpha/PLC-beta can play a significant role in scar remodeling, as well as in cardiac hypertrophy and fibrosis of the surviving tissue following AMI, suggesting that this pathway may be a new target in altering the remodeling process [12].

Arterial hypertension - The impairment of beta-adrenergic responsiveness can play a significant role in the development or maintenance of hypertension, or both. This defect has been associated with alterations in receptor/G-protein interaction. Consequently, a functional impairment of the vasodilating tonus can be significant in the pathogenesis, maintenance, or both of the peripheral vascular resistance elevated in hypertension [13].

Studies of hypertensive patients have shown an impairment of parallel beta-adrenergic vasodilatation and a reduction of adenylate cyclase activity in lymphocytes stimulated by beta-adrenergic agents. This impairment can be related to a defect in G-protein function, as suggested by some studies conducted on lymphocytes of young hypertensive patients [14].

Other studies on hypertensive and elderly patients, also using human lymphocytes, have documented the impairment of G-protein function even though unknown and controversial factors still exist. Lymphocytes of both hypertensive and elderly patients have shown a decrease of the adenylate cyclase activity stimulated by isoproterenol. However, the adenylate cyclase activity stimulated by the aluminum/fluoride complex (that stimulates directly G-proteins) was reduced only in hypertensive patients, and this effect was inversely proportional to the values of the mean arterial pressure. In young hypertensive patients, the titration of Gs-proteins with cholera toxin was significantly increased, but the immunodetected Gi-proteins did not change. On the other hand, in elderly patients, the Gs-proteins titrated with pertussis toxin were significantly increased. Unlike this last finding, the Gi-proteins titrated by immunological methods did not change [14].

It has been shown that G-proteins have a significant influence on platelet function, including abnormal activity levels in humans having non-insulin dependent diabetes. A recent study on the adenylate cyclase activity in platelet membrane preparations, where the levels of G-protein subunits were measured by immunological methods (Gs-alpha, Gi-alpha2 and beta), did not show any alteration in their values. These data do not agree with the theory that a common defect in G-proteins may explain the association of hypertension and non-insulin dependent diabetes [15].

Studies of rats demonstrated a role of the altered expression of G-proteins in the regulation of adenylate cyclase activity in the contraction force of cardiomyopathies of SHR rats. This study showed that the quantity of Gi-alpha-protein, and not only the pertussis substrate, was increased in the membranes of hypertrophic hearts of SHR rats without HF. The results of this study, using titration with the pertussis toxin, were strongly dependent on the quality of Gi-alpha substrate. The increase in the expression of this G-protein subunit and the decrease in the number of beta-adrenergic receptors reveal a possible role of these binding proteins in the regulation of adenylate cyclase activity and in the contraction force in SHR rats. Therefore, an increase in the expression of Gi-alpha can have a pathophysiological role not only in terminal HF, but also in hypertrophic cardiomyopathy as well [16].

It is known that cardiac hypertrophy induced by hypertension is a predictor of HF development. No cellular marker is known to contribute to the progress of compensated hypertrophy to HF. In HF, several defects of signal transduction lead to a desensitization of adenylate cyclase such as proven by the downregulation of beta-adrenergic receptors, increase in Gi-protein expression, and beta-adrenergic receptor uncoupling, probably due to an increase in kinase receptor activity. The majority of the studies of hypertensive heart disease have been conducted using experimental models in rats. The mechanisms in models for acquired and genetic hypertension are frequently different, but Gi-protein alteration and beta-adrenergic receptor downregulation have been often observed. These studies suggest that the subjacent mechanisms of enzymatic desensitization should be more related to the sympathetic activity as a cause of hypertension than the genetic alterations of the signal-transducing proteins. So far, the available data have suggested that a beta-adrenergic desensitization can represent a mechanism that contributes to the progression of hypertrophy to heart failure. The main question remains if those patients who develop HF are more predisposed to beta-adrenergic desensitization or if an early intervention in the reduction of the sympathetic activity is more effective in the prevention or delay of the progress of compensated hypertrophy to overt HF [17].

Through the analysis of the concepts above reviewed, it is evident that the role of G-proteins, when the cAMP system is considered, is still very obscure with extremely controversial questions, mainly when data of human hypertensive patients are considered. Although there is evidence of G-protein dysfunction, the results are divergent, revealing the nonexistence of adequate methods of study in this area.

Diabetes mellitus - In the liver, the hormone glucagon increases the concentration of cAMP by adenylate cyclase activation through a Gs-protein-mediated process. This effect of glucagon is antagonized by insulin, through molecular mechanisms that are not well-known. However, insulin receptors exhibit tyrosine kinase activity and seem to interact with G-proteins, maybe through the phosphorylation of these proteins. In type I diabetes, the circulating insulin levels are abnormally low, allowing several metabolic alterations, as well as a variety of complications such as ionic disorders, neuropathies, and respiratory and cardiovascular alterations that are predisposed to infection. Experimentally, it has been demonstrated that type I diabetes causes a loss of Gi-protein expression. As Gi can couple with receptors bound to potassium channels and exert an inhibitory effect on adenylate cyclase, it is probable that this transduction protein can be related to the alterations of type I diabetes.

Integration of information between the pathways mediated by tyrosine kinase and G-proteins is necessary for insulin action, but little is known about it. Experiments with transgenic rats show a critical participation of Gi-alpha2-protein in the insulin action. Deficiency of this G-protein in the adipose and hepatic tissues is related to hyperinsulinemia, impairment of glucose tolerance, and in vivo resistance to insulin. Resistance to insulin affects glucose transportation activity, lipolysis regulation and glycogen synthetase activation. Deficiency of Gi-alpha2-protein influences the activity of protein-tyrosine phosphatase and attenuates tyrosine phosphorylation stimulated by insulin. Deficiency of this Gi-protein creates a model of resistance to insulin characteristic of the non-insulin-dependent diabetes mellitus, implicating Gi-alpha2-protein as a positive regulator of insulin action [19].

The consequence of the metabolic alterations of diabetes is an increase in the release of vasoactive substances and proliferative agonists, which promote glomerular hyperfiltration, hypertrophy, increase of matricial deposition, and finally glomerulosclerosis. Several of these autocrine and paracrine actions are related to the receptor/G-protein coupling with an increase of the tendency to diabetic nephropathy [20,21].

The expression of tyrosine-kinase activity of the insulin receptor represents an essential step in the signal transduction of insulin through target cell membrane. Signal transduction beyond tyrosine-kinase levels is not yet understood in details. The possible mechanisms involve phosphorylation of proteic substrates, activation of serine-kinase, interaction with G-proteins, phospholipases, and phosphatidyl-kinases. Studies of multiple models of insulin-resistant cells demonstrate that an impairment of tyrosine-kinase response to insulin stimulus is a potential mechanism of insulin resistance. An impairment of the effect of insulin on tyrosine-kinase in the major insulin target tissues, particularly skeletal muscle, was demonstrated in type II diabetic patients (non-insulin dependent). There is no evidence that the functional alteration of tyrosine-kinase in the skeletal muscle is a primary defect. However, it is very probable that an abnormality in signal transduction of the insulin receptor by G-proteins significantly contributes to the pathogenesis of insulin resistance in type II diabetes [22].

In regard to vascular alterations in diabetic patients, experimental models failed to provide definitive data. Thus, it is not possible to state that these functional alterations are related to alpha-adrenergic receptors and their coupling G-proteins. Experiments using immunological methods did not show differences between Gi2, alpha3-proteins, and Gq11alpha-proteins among controls, the aorta, and the caudal artery of diabetic rats. Therefore, alterations in number of alpha1-receptors or G-protein level cannot alone explain the increase of the contractile response of diabetic arteries through noradrenaline action. Although there is no definitive proof, perhaps due to methodological limitations, it is expected that an increase in G-protein activity coupled with alpha1-receptors and with phosphatidylinositol/phospholipase C cycle can be related to an increased response induced by alpha1-adrenergic stimulation in diabetic arteries [23].

Dyslipidemia - The regulation of cholesterol intracellular transportation is mediated not only by extracellular concentration of lipoproteins and transcriptional responses to alterations in intracellular content of free cholesterol. In addition to these factors, the modulation of cholesterol transportation is also regulated by products synthesized after the activation of signal-transducing pathways originated in the cellular surface. These factors have been identified, and the importance of the generation of secondary messengers, especially eicosanoids and cyclic AMP, which influence the specific effects of proatherogenesis [24], has been demonstrated.

Atherosclerosis - There certainly are mechanisms related to the adenylate cyclase activity in atherosclerosis, but they are, so far, unknown. An interesting study in humans, using membranes of erythrocytes of patients with chronic ischemic heart disease, relates alterations of Gs- and Gi-proteins to coronary atherosclerosis. Patients with decreased Gs-proteins had Gi-proteins almost normal, and those who presented increased Gi-proteins did not show significant alterations of Gs-proteins. Patients with increased Gi-proteins (Gs normal) showed a more severe deterioration of their coronary arteries than those with decreased Gs-proteins (Gi normal). As these two groups

did not present significant differences in serum lipid levels, hormones, medication, and clinical history data, it seems evident that there is participation of G-proteins, more specifically a dysfunction of Gi-proteins, in atherosclerosis. In addition, the generation of secondary messengers, especially eicosanoids and cyclic AMP, can have specific pro-atherogenic effects [25].

Heart failure - Congestive heart failure is associated with inotropic and chronotropic hyporesponsiveness to adrenergic stimulation, with decrease of Gs-alpha-proteins, increase of Gi-alpha-proteins, and a reduction in adenylate cyclase activity [26].

In HF, there is a strong sympathetic activation, which causes a reduction of beta-adrenergic activity in these conditions. In regard to membrane receptors, there is a downregulation of beta1-receptors and uncoupling of beta2-receptors. Unlike upregulation of Gi-proteins, there is no alteration in Gs-alpha-protein levels and betagama subunits. The increase of Gi-proteins alone can suppress adenylate cyclase activity even in the absence of downregulation of beta-adrenergic receptors. As cardiac hypertrophy is a strong predictor of HF, these observations indicate that the desensitization of adenylate cyclase by Gi-proteins can be a significant pathophysiological mechanism in the progression of compensated cardiac hypertrophy to HF [27]. In addition, similar alterations can be observed with aging [28].

Major efforts have been devoted to understanding how pathophysiological conditions, such as ischemia and CHF, and the therapeutical methods used to treat these conditions alter or regulate receptor systems. It is very clear that the problem is not restricted to the number of receptors; it is also related to interactions between them and G-proteins. Recent evidence suggests that CHF in humans is associated with a decrease in beta-receptors and increase in the quantity of Gi-protein. Understanding alterations can bring significant knowledge to the development of new therapeutical methods. For example, treatment with a beta-blocker with intrinsic mimetic activity (metoprolol) restores reduced levels of beta-receptor density. Studies using biopsies of patients with CHF showed that the use of metoprolol, unlike the traditional treatment with digitalis, diuretics, and angiotensin converting enzyme inhibitors, led to a significant reduction of 74% of Gi-proteins and a selective increase of beta1-receptors [29]. Therefore, we can conclude that the possible beneficial effects of beta-bloto with a partial reversion of Gi-protein upregulation and beta-receptor downregulation.

G-proteins, the cyclic GMP system and cardiovascular diseases

The cyclic GMP system began to be exhaustively studied after the experimental conclusion was reached that EDRF was NO, which when spread to smooth muscle caused vasodilatation by stimulating guanylate cyclase. Later studies established a direct link between endothelial dysfunction and the pathophysiology of cardiovascular diseases.

Myocardial ischemia – After global myocardial ischemia followed by reperfusion, coronary arteries loose their ability to express vasodilatation depending on endothelium and mediated by receptors. On the other hand, the endothelium-dependent relaxation mediated by ionophore calcium A23187, which does not depend on receptor stimulation, is unaltered. In addition, the endothelium-dependent vasodilatation produced by exogenous phospholipase C is also normal, but the relaxation produced by sodium fluoride, which acts through G-proteins that are sensitive to the pertussis toxin is impaired. These data indicate that the impairment in the production of EDRF/NO mediated by receptors following reperfusion lesion can be due to a G-protein dysfunction that binds the endothelial cell receptors to the EDRF/NO synthesis pathway. When endothelial dysfunction is studied in cardiovasculopathies, a common link can be found, similar to that described in the experiments with the global ischemia model followed by reperfusion. Similar to what was observed in this model, a pattern of functional impairment of receptors and of the signal transduction represented by G-proteins can be noted. On the other hand, the cell capacity to produce EDRF/NO remains unaltered, as well as the function of vascular smooth muscle. This pattern of impairment is common to arterial hypertension (AH), dyslipidemia, atherosclerosis, diabetes mellitus, Raynaud's phenomenon, HF, AMI, etc [5,6,8].

Arterial hypertension - NO stimulates ADP-ribosylation of cytosolic and membrane proteins. ADP-ribosyltransferase alters several intracellular and membrane proteins, including G-proteins. With ADP-ribosylation of the G-proteins of vascular smooth muscle, there is activation of adenylate cyclase and reduction of phospholipase C, leading to vasodilatation. In AH, the chronic reduction of cGMP activity with a decrease in NO release leads to a reduction in G-protein activation and an increase in sensitivity to vasoconstricting agonists [30].

Diabetes mellitus - Vascular reactivity in diabetic models is impaired, with a higher possibility of diffuse atherosclerotic disease. The release of NO is decreased, allowing the inference, as in other models of cardiovascular disease, that there is impairment of signal transduction at the level of receptor/G-protein coupling in the cGMP system, as occurs in the cAMP system. Glucose stimulates in vivo and in vitro the secretory and mitogenic activities of beta cells of the pancreas that produce insulin. The mechanisms of this action remain little understood. It is known that glucose stimulates replication, insulin secretion, and cAMP formation. These effects can be imitated by cAMP agonists depending on protein kinase, but not by cGMP. The pre-treatment of pancreatic islets with pertussis toxin, which regulates signal transduction through G-proteins related to the cGMP system, completely inhibits

the stimulatory effect of glucose on the mitogenesis of beta cells of the pancreas, but does not inhibit the secretion of insulin. Thus it is possible to conclude that the activation of protein kinase C or the synthesis of cAMP is enough to increase mitogenesis and insulin secretion, while cGMP does not affect these processes. There is experimental evidence that cAMP does not take part in the mitogenesis and in the secretory action of glucose. On the other hand, it seems that signal transduction through G-proteins and activation of protein kinase C is necessary to the message induced by sugar in regard to mitogenesis, but not to the secretory activity of beta cells of the pancreas [31].

In a model for type I diabetes (insulin-dependent) produced by streptozotocin in rats, the alteration of the mechanisms of signal transduction in the retina has been demonstrated since the earliest stages. Experimental data are strongly suggestive of G-protein deterioration, especially Gi (sensitive to pertussis toxin), in diabetic retina [32].

The alteration of vascular reactivity and predisposition to atherosclerosis related to deficient production of NO in diabetes are very well known. As this mediator acts through cGMP, the participation of the G-proteins involved in this system in the pathogenesis of diabetes is evident.

Dyslipidemia - The explanations for impairment of endothelium-dependent vascular relaxation in hypercholesterolemia include alterations in signal transduction, deficiency in the substrate (arginine) of the enzyme NO synthetase, alterations of this enzyme or one of its co-factors, and excessive destruction of NO by the superoxide anion.

The lysophosphatidic acid is a natural phospholipid that affects the intracellular signaling pathways in situations of vascular wall alterations that can precede the onset of atherosclerosis. This phospholipid causes an increase in cytosolic calcium in the presence or absence of extracellular calcium, strongly stimulates the change Na+/H+ and the mitogenesis-activated protein kinase. These effects are blocked by pertussis toxin, demonstrating that the effects of the lysophosphatidic acid on signal transduction pathways and on the action of growing factors are mediated by G-proteins [33].

Another significant fact is that hypercholesterolemia is associated with a reduced guanylate cyclase response through the action of nitrovasodilators in smooth muscle [34].

Atherosclerosis - In atherosclerosis and associated conditions, the importance of a defect in endothelial signal transduction is demonstrated by impairment of endothelium-dependent relaxations. These relaxations, which are sensitive to pertussis toxin, are markedly altered in experimental atherosclerosis in pigs. Gi-protein expression is decreased in atherosclerosis and associated conditions in human coronary arteries. Therefore, the dysfunction of Gi-proteins (sensitive to pertussis toxin) can contribute to impairment of endothelium-dependent relaxation in atherosclerosis [35].

Pertussis toxin selectively promotes ADP-ribosylation of certain G-proteins (specially Gi). In the model that uses porcine coronary arteries, this toxin inhibits the release of NO induced by certain agonists (serotonin, alpha2-adrenergic agonists, leukotrienes, thrombin), but not all (bradykinin, ADP), endothelium-dependent vasodilators, suggesting that both Gi and Gq-proteins can be coupled with activated receptors to increase the cytosolic calcium concentration necessary for NO synthetase stimulation. In arteries with regenerated endothelium and in endothelial cell culture, NO release induced by pertussis toxin-sensitive mechanisms is markedly reduced or absent, but the response to other agonists is normal. Based on these experiments, it is possible to consider that a Gi-protein abnormal function, more than a reduction, or a reduction in membrane receptor sensitivity can predispose vessel wall to vasospasm and onset of the atherosclerotic process [36].

Lysophosphatidylcholine can activate protein kinase C in intact vessels, leading to an increase of superoxide radical production. This activation can also alter NO release in response to acetylcholine. Thus, as NO is closely related to the cyclic GMP system and its G-proteins, it is worth stressing that the reduction of atherosclerotic vessel relaxation can be related to a greater production of superoxide radicals, with important consequences in the atherosclerotic process [37].

Heart failure - NO inhibits the positive inotropic action to beta-adrenergic stimulation in humans with left ventricular dysfunction due to idiopathic dilated cardiomyopathy, but not in individuals without HF [38,39].

In HF, in addition to the already mentioned impairment of vascular relaxation mediated by cAMP, there is also impairment of relaxation mediated by cGMP (NO). In HF, the spontaneous release of NO is preserved or increased, while the stimulated release, including during exercise, is impaired. Furthermore, there is the possibility that myocardial NO production, as an activity of cytokines and expression of the inducible form of NO synthetase (NOSi), can be related to a contractile defficiency [39].

The specific bibliography, as extensive as possible, relating HF, cGMP system and G-proteins, does not reveal works with specific mention to signal transduction proteins. However, as there are innumerable works (much fewer than the ones related to the cAMP system) showing functional alterations associated with the cGMP system, one can state with reasonable safety that there is impairment of G-protein signal transduction. It can also be speculated that this function can be normal during rest and impaired during exercise or other conditions of beta-adrenergic or cholinergic stimulation. This speculation is based on the alterations of impairment of NO release and on the similarity of the behavior of Gs-proteins in ischemic and dilated cardiomyopathies, where they are reduced [40-42].

Conclusion

The crucial role that G-proteins have on transmembrane signal transduction has been emphasized by the rapid

expansion of the list of receptors and effector molecules, which are coupled by G-proteins. These proteins are equalized to allow discrimination and diversification of cellular signals in cytosolic medium. The use of an evolutionarily preserved "GTPase watch" by G-proteins implies the knowledge of the basic biological role that these proteins may play. The knowledge of this altered expression or function of G-proteins in human diseases is an ever-emerging subject. It is not surprising that the deficiency of expression or altered forms of these important proteins may lead to global or restricted metabolic disorders, depending on the distribution and role of G-proteins. Human diseases including alcoholism, endocrine diseases, neoplasias, and cardiovascular diseases discussed in this text are currently recognized as partial consequences of the impairment of expression or function of G-proteins[43].

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