

# Beta-Adrenergic Pathway in Healthy and Hypertrophied Hearts

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## History

Interest in the mechanisms responsible for cellular response to determined extracellular stimuli has long been a reason for intense scientific investigation<sup>1-5</sup>. Langley<sup>1</sup>, in 1905, was the first author to propose that agents, when acting upon nervous terminations, do not interact directly with the cells but with receptor substances, which are cellular response mediators. In 1913, Ehrlich<sup>3</sup> used the term receptor to designate a specific chemical group reacting to a determined drug. In 1948, Ahlquist<sup>4</sup> suggested that adrenergic stimulation interacted with two types of receptors, alpha ( $\alpha$ ) and beta ( $\beta$ )-adrenergic receptors. It was Kahn<sup>5</sup>, however, who in 1976 best defined the term receptor as being a molecule or molecule complex able to recognize and interact with hormone, drug or neurotransmitter and, after this interaction, generate a signal capable of starting a chain of events resulting in a biologic response.

## Signal transduction – the $\beta$ -adrenergic pathway

An agonist binding to a receptor, followed by conversion of extracellular stimulus to intracellular response, is called signal transduction<sup>1,2,6</sup>. In regard to catecholamines and  $\beta$ -adrenergic receptors, this transduction has usually been called signaling or the  $\beta$ -adrenergic pathway.

Extracellular stimulus generated by the autonomic nervous system (ANS) (1<sup>st</sup> messenger) acts upon the receptor (agonist/receptor binding), mediated by the binding protein (G-protein), interacts with the effector (adenylate cyclase), activating or inhibiting the production of adenosine 3', 5' cyclic monophosphate (cAMP) (2<sup>nd</sup> messenger). This sequence of events causes changes in the enzymes and ion channels, triggering, among other responses, alterations in the metabolism, mainly in the transport of cytosolic  $\text{Ca}^{2+}$  (fig.1).

Biologic (or cellular) response, mediated by receptors and effectors located in the external and internal layers of the sarcolemma, increases or decreases, respectively, under the influence of the adrenergic or cholinergic ANS (fig. 1).

## Components of the $\beta$ -adrenergic pathway

The  $\beta$ -adrenergic pathway consists of  $\beta$ -adrenergic receptors, activating binding protein (Gs), adenylylase and cAMP.

**$\beta$ -Adrenergic receptors** - They consist of two subtypes,  $\beta_1$  and  $\beta_2$ . Cardiac  $\beta$ -adrenergic receptors are predominantly of the  $\beta_1$  subtype, and the noncardiac ones, such as those of vessels and lungs, are of the  $\beta_2$  subtype<sup>7</sup>. In humans, the predominant population of ventricular receptors is  $\beta_1$ ;  $\beta_2$  corresponds to only 20%<sup>8,9</sup>. The number of  $\beta_2$  receptors in the atria, sinus and atrioventricular nodes is twice that of the ventricles<sup>10</sup>. Although the coupling degree of  $\beta$  receptors to adenylylase via G-protein is four to five times greater in the  $\beta_2$  subtype<sup>11-13</sup>, and their affinity to agonists is 40 to 50 times greater in the subtype  $\beta_1$ <sup>14</sup>, the intensity of response of the  $\beta$ -receptors to the agonist's action is directly proportional to the number of receptors<sup>8</sup>. In the heart, the action of  $\beta_1$  receptors is inotropic, while the action of  $\beta_2$  receptors is chronotropic and dromotropic<sup>1,2,8</sup>. Even though different,  $\beta_1$  and  $\beta_2$  receptors have some similarity in their molecular structures. This similarity explains why specific agonists or antagonist agents, for instance those of the  $\beta_1$  receptor, when used in high doses, lose specificity and start to act also upon  $\beta_2$  receptors.

**G-proteins** - G-protein is a crucial binding protein in the interaction of the  $\beta$ -receptor with the adenylylase effector to cAMP formation. Adenylylase activity is modulated by two G-proteins: Gs, capable of stimulating, and Gi, capable of inhibiting adenylylase activation<sup>15</sup> (fig. 1). Gs protein is formed by the  $\alpha_s$ ,  $\beta$  and  $\gamma$  subunits; in its inactive form, the  $\alpha_s$  subunit is coupled with guanosine diphosphate (GDP). After the agonist action upon the  $\beta$  receptor,  $\alpha_s$  exchanges GDP for guanosine triphosphate (GTP), separates from  $\beta$  and  $\gamma$  subunits and interacts with adenylylase, which when activated, forms cAMP. By the action of an enzyme, guanosine triphosphatase (GTPase), the  $\alpha_s$  subunit exchanges GTP for GDP, becoming inactive again (fig. 2). This same  $\alpha_s$  subunit, in addition to

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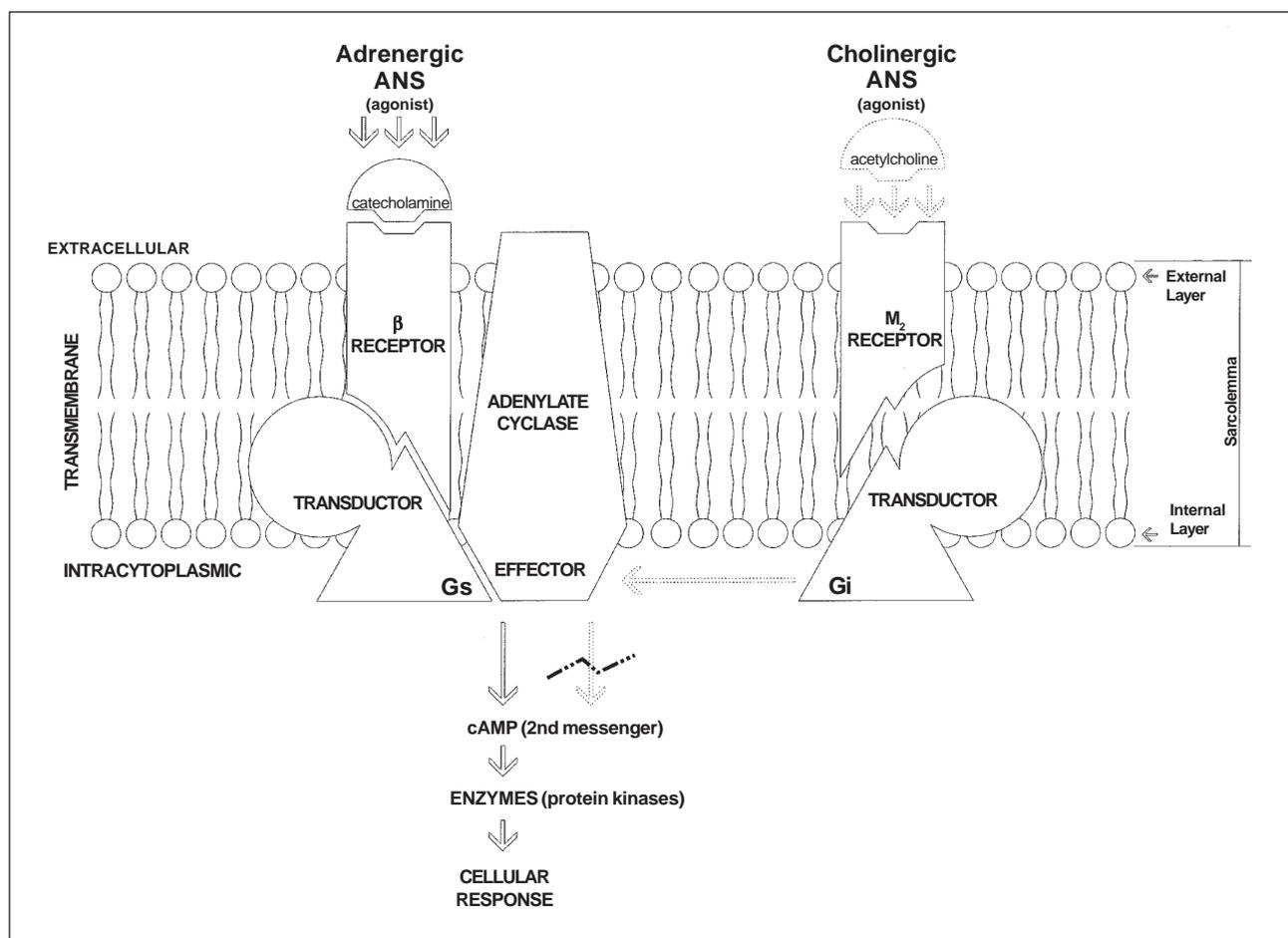


Fig. 1 – Signal transduction. It starts with agonist binding to receptor, converting the extracellular stimulus to biologic effect or cellular response. ANS – autonomic nervous system;  $\beta$  – beta-adrenergic receptor;  $M_2$  – muscarinic receptor; Gs – stimulating G-protein; Gi – inhibiting G-protein; cAMP – 3', 5' cyclic adenosine monophosphate. Modified from Fleming et al Circulation 1992; 85: 420.

activating adenylyl cyclase, also promotes direct activation of the calcium channels of the sarcolemma<sup>1,2</sup> (figs. 3 and 4). Gi protein, formed by the same  $\alpha$ ,  $\beta$  and  $\gamma$  subunits after the agonist action of acetylcholine upon the muscarinic receptor, promotes inhibition of the adenylyl cyclase activity. In its inactive form, the  $\alpha_i$  subunit is found bound to GDP. Like Gs, in exchanging GDP for GTP, the  $\alpha_i$  subunit separates from the  $\beta$ - $\gamma$  subunits, becoming the active Gi protein. However, unlike Gs, the  $\beta$ - $\gamma$  subunits, when stimulating GTPase, decrease the  $\alpha_s$ -GTP binding, promoting inhibition of the adenylyl cyclase activity<sup>1,2</sup>. These same subunits also stimulate phospholipase  $A_2$ , which in turn activates potassium channels, causing membrane hyperpolarization and, hence, heart rate reduction<sup>1,2</sup>. G-proteins have a major role in determined cardiovascular situations and diseases. For instance, while long-term treatment with thyroid hormone and physical training causes an increase in Gs protein<sup>16</sup>, in dilated cardiomyopathy and heart failure, a decrease in Gs protein and elevation in Gi protein occur<sup>17</sup>.

**Adenylyl cyclase** – Adenylyl cyclase is the only protein producing cAMP and, for doing this, it needs only ATP and magnesium. The enzyme adenylyl cyclase has a structure similar to that of the calcium channels, and it

is commonly found in the inner layer of the sarcolemma. However, it may also exist in the sarcoplasmic reticulum (SPR)<sup>18-21</sup>. Usually, adenylyl cyclase is activated by the stimulation of  $\beta$ -adrenergic receptors; however, it may also undergo direct action of forskolin<sup>22</sup> or be activated by the stimulation of other specific receptors, such as histamine ( $H_2$ ), dopamine ( $DA_1$ ), glucagon and prostacyclin.

**cAMP** – cAMP plays a crucial role in the activation of the protein kinases. These proteins are enzymes responsible for activation and deactivation of ion channels and intracellular organelles (fig. 4). Protein kinases, which are normally found in their inactive form, are constituted by two subunits, one regulator (R) and another catalytic (C). cAMP interacts with the inactive protein kinase, binds to the R subunit and releases the C subunit, activating it. cAMP is degraded by phosphodiesterase via calmodulin kinase, an enzyme activated by elevating the concentration of cytosolic  $Ca^{2+}$ <sup>1,2</sup> (figs. 3 and 4). A fast, dynamic and constant balance occurs between cAMP formation and degradation. Thus, variations in its amount in different tissues are mainly related to the  $\beta$ -agonist action of the catecholamines.

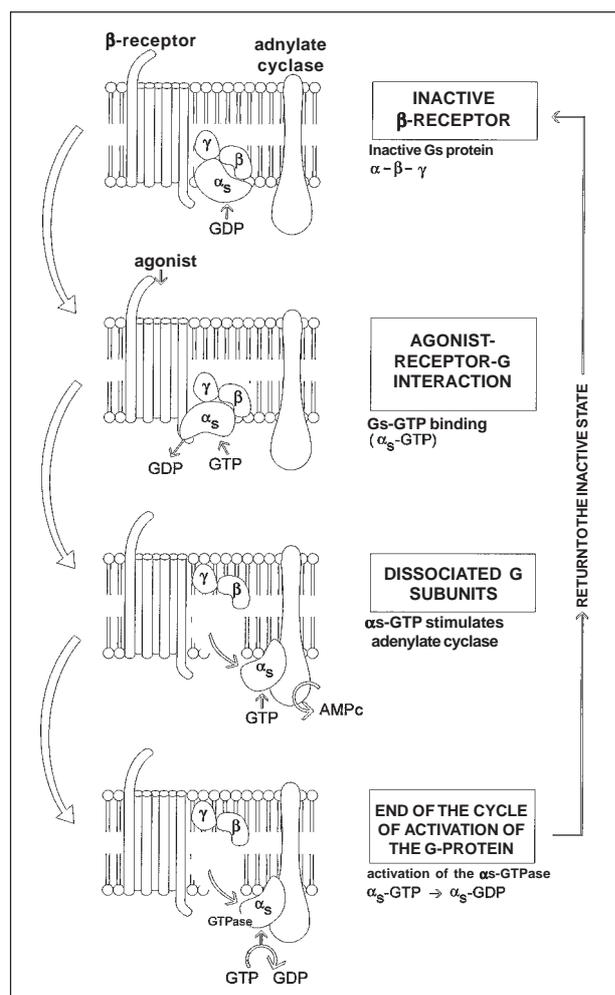


Fig. 2 – Gs protein activation and deactivation cycle. Under the agonist action,  $\beta$ -receptor promotes GDP exchange for GTP bound to the  $\alpha_s$  subunit.  $\alpha_s$ -GTP interacts with adenylylase, inducing the formation of cAMP. By GTPase action, the  $\alpha_s$  fraction exchanges GTP for GDP, returning to its inactive state. GDP-guanosine diphosphate; GTP- guanosine triphosphate; GTPase: guanosine triphosphatase. Modified from Neer EJ<sup>15</sup>.

## $\beta$ -adrenergic pathway activation

$\beta$ -adrenergic receptor, inactive in the membrane after undergoing the action of the agonist agent (agonist-receptor interaction), promotes the exchange of GDP for GTP, activating the Gs protein. The  $\alpha_s$  fraction interacts with adenylylase<sup>15</sup>, inducing the formation of cAMP (fig. 2). It is also important to remember that cAMP is indirectly activated by  $\beta$ -adrenergic stimulation that, in promoting the elevation of  $Ca^{2+}$  concentration in cytosol, activates calmodulin kinase and, hence, phosphodiesterase, causing cAMP degradation<sup>1,2</sup>.

## Desensitization and downregulation of $\beta$ -adrenergic receptors

Under the continuous action of  $\beta$ -adrenergic agonist, cAMP activates a protein kinase,  $\beta$ -adrenergic receptor kinase ( $\beta$ -ARK)<sup>22</sup>, which, in phosphorylating the receptor,

inactivates it, causing uncoupling of the receptor – Gs – adenylylase complex<sup>23-30</sup>. Uncoupled from the effector, the receptor passes into the intracytoplasmic space, momentarily diminishing the number of receptors available in the membrane. In addition to  $\beta$ -ARK, the major role of arrestins should be remembered, mainly  $\beta$ -arrestin 1, in the process of uncoupling and internalization of  $\beta$ -adrenergic receptor. The  $\beta$ -arrestins are proteins that bind to the G-protein coupled receptor<sup>31-33</sup>. This phenomenon, usually called desensitization, (fig. 5), causes a reduction in the response to  $\beta$ -adrenergic stimulation promoted by hormones or neurotransmitters. The  $\beta$ -receptor, once internalized under the effect of phosphatase, is dephosphorylated, becoming able to be reincorporated into its original place in the membrane, a phenomenon called resensitization<sup>25</sup> (fig. 5).

Therefore, in myocardium, the  $\beta$ -adrenergic receptors have a round-trip itinerary between their location in the membrane and the intracytoplasmic space<sup>29</sup>. This mechanism alters heart sensitivity, allowing the heart to respond with greater or lesser intensity to determined stimuli. However, in the intracytoplasmic space, the  $\beta$ -receptor may be consumed, a phenomenon called sequestration, which causes a decrease in the number of cellular receptors. Thus, the density of receptors, i.e., the number of receptors per sarcolemma unit, is not constant; it can diminish or increase in physiologic circumstances or pathologic conditions. These variations are respectively called downregulation and upregulation. Although the desensitization and downregulation phenomena are well defined, the use of the term downregulation remains controversial. While some authors use the term to refer to receptor desensitization, others more correctly suppose that downregulation would implicate true changes in the total number of receptors<sup>1</sup>. This decrease results from receptor internalization, sequestration and consumption by lysosomal or nonlysosomal mechanisms<sup>34</sup> and/or decrease in the velocity of the synthesis of the receptor<sup>35-37</sup>. With a smaller number of receptors, the cardiac cell may lose or have a diminished ability to respond to agonist action.

## $\beta$ -adrenergic effects

At the subcellular level, many cAMP effects are mediated by protein kinases. These kinases promote protein phosphorylation, causing activation and deactivation of different enzymes involved in the cellular metabolism of lipids and carbohydrates, in the citrate cycle, and mainly in the regulation of the cytosolic calcium transport. Activation or deactivation of enzymes causes a variety of biologic effects, resulting in changes in cardiac muscle properties. The effects have usually been called  $\beta$ -adrenergic effects<sup>1,2</sup> (fig. 3).

Gs protein activated by the agonist binding to the  $\beta$ -adrenergic receptor, in addition to stimulating adenylylase by inducing the formation of cAMP, also acts directly in the  $Ca^{2+}$  channels of the sarcolemma, promoting an increase in its permeability. The protein kinase A (PKA), activated by cAMP, promotes phosphorylation of the sarcolemma calcium channels, troponin I and phospholamban.

The direct action of the Gs protein on the sarcolemma calcium channels and phosphorylation of the calcium channels, via cAMP, promote an increase of Ca<sup>2+</sup> concentration in cytosol, resulting in a positive inotropic effect. Phosphorylation of phospholamban promotes liberation, activates the SPR calcium pump, causing greater and faster Ca<sup>2+</sup> uptake by SPR<sup>38</sup>, which promotes improvement in relaxation, that is, a lusitropic effect<sup>39</sup>. However, it is important to remember that phospholamban phosphorylation also occurs by Ca<sup>2+</sup>-calmodulin kinase action<sup>40</sup>, an enzyme that is activated when there is an elevation of Ca<sup>2+</sup> concentration in cytosol. On the other hand, the phosphorylation of troponin I promotes decrease in the sensitivity of the calcium contractile system, which induces an increase in the velocity of cellular relaxation<sup>1,2,41</sup> (figs. 3 and 4).

When the β-adrenergic action is exerted upon the sinus node, it promotes a chronotropic effect and when exerted upon the atrioventricular node, His' bundle and Purkinje's fibers it causes a dromotropic effect. The chronotropic effect, which corresponds to an increase in the velocity

of the generation of stimuli, is translated by an increase in the number of heartbeats. The dromotropic effect, which means an increase in the velocity of impulse conduction, corresponds to shortening of the PR space in the electrocardiogram, and shortening of the AH and HV intervals in the His' bundle electrogram.

### Cardiac hypertrophy

In normal conditions, there is a balance between the workload imposed on the heart and the amount of cardiac mass. When this balance is broken due to abnormal overload, the heart responds with the development of hypertrophy. Depending on the characteristics of the overload imposed (type, intensity and installation mode) and the animal undergoing the overload (age, gender and species), the hypertrophied tissue may show normal or altered biologic properties (in RNA, protein and myosin synthesis, in energetic metabolism and mainly in the intracellular Ca<sup>2+</sup> cycle)<sup>42-45</sup>.

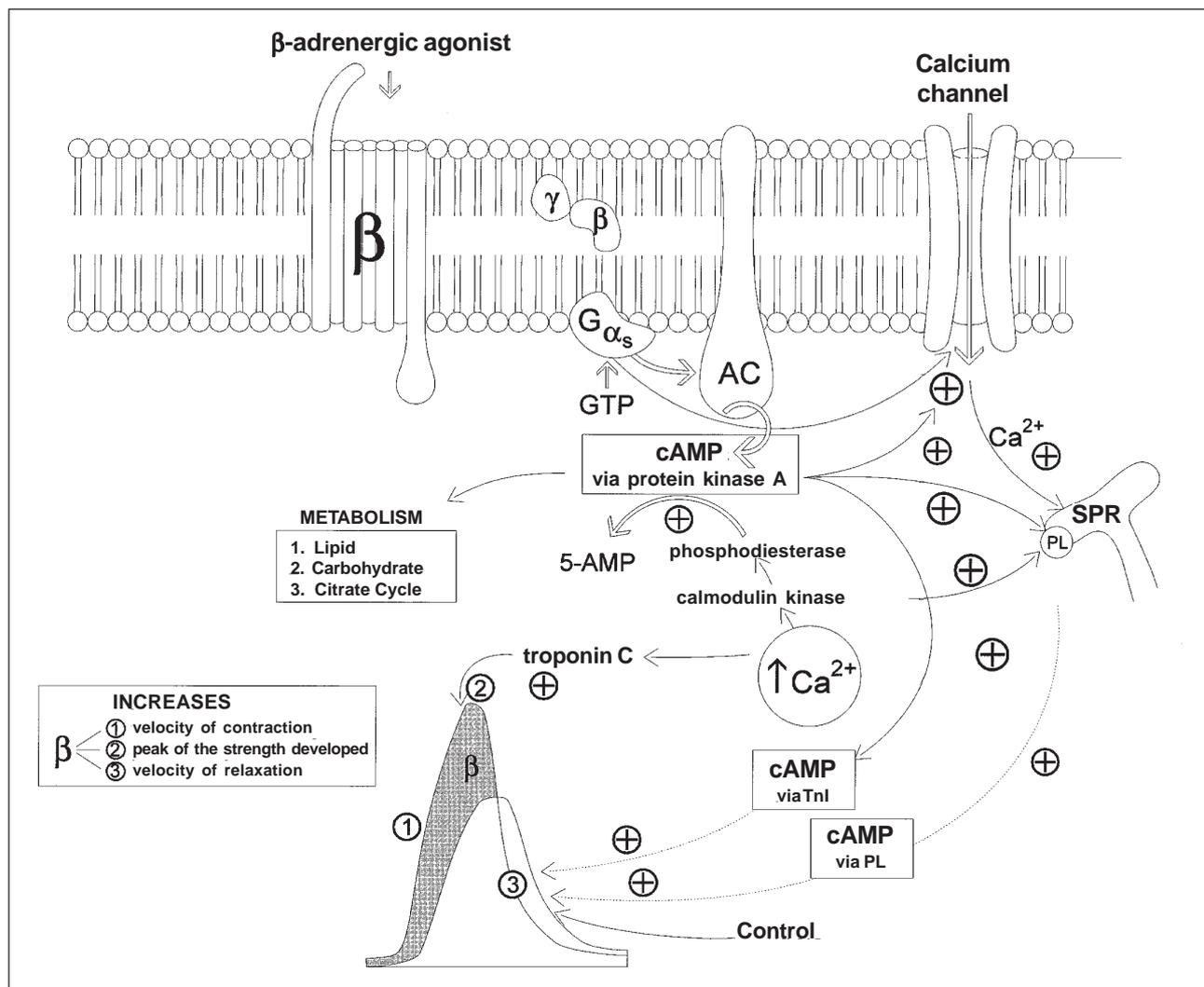


Fig. 3 – Activation of the β-adrenergic pathway and physiological beta-adrenergic effects on myocardium. SPR- sarcoplasmic reticulum; cAMP- 3',5' cyclic adenosine monophosphate; PL- phospholamban. Modified from Opie LH<sup>2</sup>.

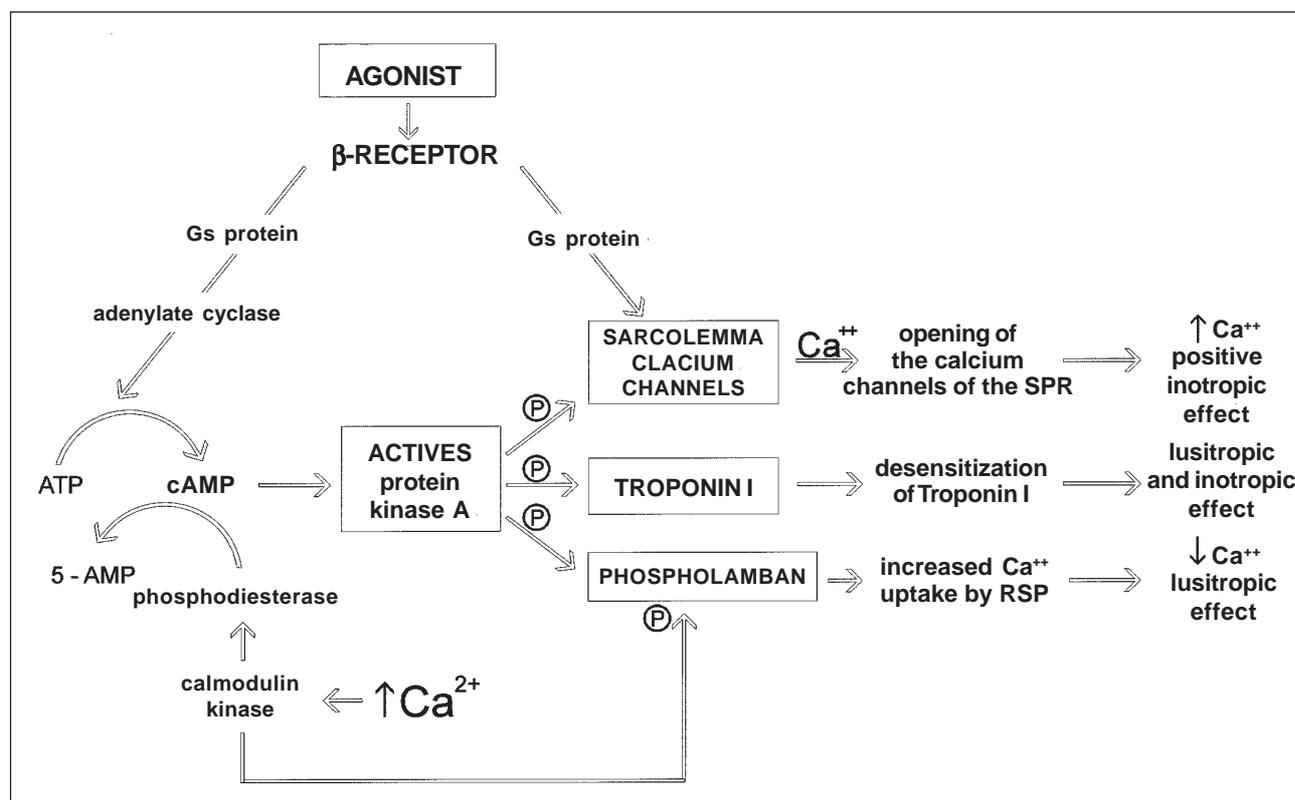


Fig. 4 – Schematic representation of the organelles dependant on cAMP action and on direct Gs protein action. P- phosphorylation. Modified from Opie LH<sup>2</sup>.

Cardiac hypertrophy should not be understood only as an expansion of the contractile complex, because the hypertrophy process is characterized by an increase in the synthesis of ribonucleic acid (RNA), proteins and myosins, and induction of new genetic expressions of protein synthesis<sup>42</sup>. Therefore, as proteins, the components of the  $\beta$ -adrenergic pathway may undergo changes during the development of cardiac hypertrophy.

In hypertrophied hearts from individuals or animals with signs of heart failure, there is depression of the functional response to sympathomimetic drugs and to direct adenylate cyclase stimulation by forskolin<sup>40</sup>. There are also alterations of the components of the  $\beta$ -adrenergic pathway<sup>46,47</sup> (table I), which vary according to the kind of heart disease<sup>48</sup> (table II). On the other hand, in patients or animals with stable hypertrophy, that is, without signs of heart failure, the results are controversial. Although some authors have not described changes in mechanical response to sympathomimetic stimulation<sup>49,50</sup> and others have observed alterations only in the contractile phase<sup>51</sup>, most of the works show depression of responses of the contractile and myocardial relaxation phases during  $\beta$ -adrenergic stimulation<sup>52-59</sup>.

Although the  $\beta$ -adrenergic pathway seems to be one of the main mechanisms responsible for this depression in the response of hypertrophied cardiac muscle<sup>53,55-60-69</sup>, other intracellular factors may also be involved, such as  $Ca^{2+}$  transport at the cellular level, PKA,  $Ca^{2+}$ -calmodulin

kinase and phosphodiesterase activities, or even the troponin C affinity for calcium<sup>51,70</sup>.

Alterations in the  $\beta$ -adrenergic pathway components, observed in the stable hypertrophied cardiac muscle, also do not agree: 1)  $\beta$ -adrenergic receptors: maintenance, increase and decrease in  $\beta$ -receptor number and affinity have been found in different kinds of experimental cardiac hypertrophy models<sup>43,46,53-55,60</sup>. Thus, while Cervoni et al<sup>54</sup> observed a decrease in number and an increase in affinity of the  $\beta$ -receptors in aorta-coarcted rats for 28 days, Limas et al<sup>61</sup> found an increase in number and a decrease in affinity of these receptors in aorta-coarcted dogs. On the other hand, Atkins et al<sup>51</sup> and Foster et al<sup>62</sup> did not find any variation in the number and affinity of receptors, respectively in spontaneously hypertensive and aorta-coarcted rats, despite de-

Agonist	Contractile response	$\beta$ -adrenergic way components	Alterations
Calcium	↔	N of $\beta_1$ receptors /mRNA ( $\beta_1$ )	↓↓ / ↓↓
Forskolin@	↔	N of $\beta_2$ receptors /mRNA ( $\beta_2$ )	↔ / ↔
$\beta_1$ agonist	↓	Gs protein/mRNA (Gs)	↓ / ↔
$\beta_2$ agonist	↓	$\beta$ -ARK/ mRNA ( $\beta$ -ARK)	↑ / ↔ ↑↑ / ↑↑

$\beta$ -ARK- beta adrenoreceptor kinase; N- number; ↔: no alteration; ↑↑ : increased; ↓↓ : decreased. (Modified from Bristow MR<sup>46</sup>).

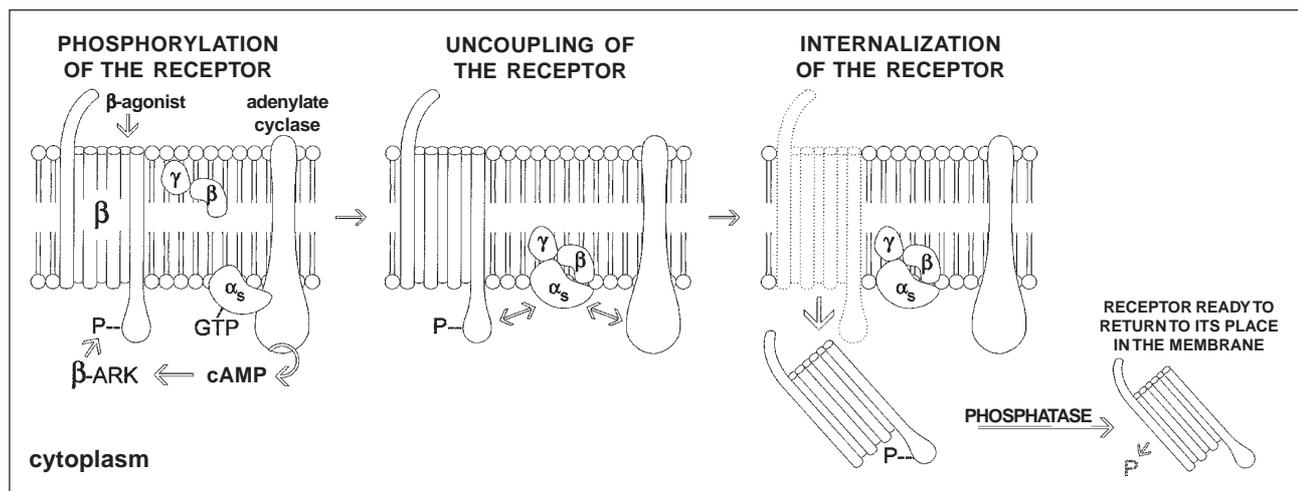


Fig. 5 –  $\beta$ -adrenergic receptor desensitization and “resensitization”. cAMP elevation stimulates  $\beta$ -ARK, which phosphorylates the receptor, inactivating it. Inside the cytosol, the receptor is dephosphorylated by phosphatase action, being able to return to its place in the membrane.  $\beta$ - beta-adrenergic receptor; GTP- guanosine triphosphate; cAMP- 3', 5' cyclic adenosine monophosphate;  $\beta$ -ARK- beta-adrenoreceptor kinase; P- phosphorylation. Modified from Lefkowitz RJ<sup>23</sup>.

Table II - Abnormality of the components of the  $\beta$ -adrenergic pathway in different types of heart disease

Abnormality of $\beta$ -adrenergic pathway components	Types of Heart Disease		
	Idiopathic (LV+RV)	Ischemic (LV+RV)	Primary PH (RV)
Reduction of $\beta_1$ -adrenergic receptor number	++	+	+++
Reduction of $\beta_2$ -adrenergic receptor number	—	—	—
Increase of Gi protein activity	+	+	—
Reduction of Gs protein activity	+	—	—
Uncoupling of $\beta_2$ -adrenergic receptor	+	+	+
Uncoupling of $\beta_1$ -adrenergic receptor	—	+++	—
Reduction of adenylate cyclase activity	+(RV)	—	+++

PH- pulmonary hypertension; LV- left ventricle; RV- right ventricle; —: no abnormality; + discrete abnormality; ++: moderate abnormality; +++: accentuated abnormality. Modified from Bristow MR<sup>48</sup>.

pression of the functional cardiac response, when stimulated by isoproterenol and forskolin @. Therefore, contractile response depression would not be related to receptors but to some of the remaining likely mechanisms previously quoted; 2) G-proteins: while Mondry et al<sup>66</sup> did not find any alteration in Gs and Gi proteins in the hypertrophied heart of aorta-coarcted rats, Nakamura et al<sup>67</sup> observed a reduction in the level of mRNA and activity of Gs and Gi proteins in hamsters with genetic myocardial hypertrophy and in aorta-coarcted rats. Kumano et al<sup>64</sup> found a decrease only in the Gs protein activity in the hypertrophied hearts of spontaneously hypertensive rats or those with renovascular hypertension. Holmer et al<sup>68</sup>, however, studying the hearts of rats undergoing aortic supravulvar bandage, and Böhn et al<sup>69</sup>, studying patients with hypertensive heart disease, observed depression of the Gs protein activity and exacerbation of the Gi protein; 3) adenylate cyclase/cAMP: most of the authors, also in different experimental hypertrophy models, describe depression of the adenylate cyclase activity and reduction of the cAMP formation in the absence of alterations in the number and/or affinity of  $\beta$ -adrenergic receptors<sup>62,63,65</sup>. Disagreement about the alterations of the already described  $\beta$ -adrenergic pathway components was also

observed by Kumano et al<sup>64</sup>, who, studying different experimental cardiac hypertrophy models, observed distinct biochemical defects of the  $\beta$ -adrenergic pathway. Thus, a summary of the most likely alterations of the different components of the  $\beta$ -adrenergic pathway may be seen in table III.

Summarizing, analysis of the literature allows concluding that, in stable cardiac hypertrophy, the mechanical behavior of the muscle resulting from  $\beta$ -adrenergic stimulation is depressed. However, there are some controversies regarding the participation of different components of the

Table III – Alterations of the components of the  $\beta$ -adrenergic pathway in stable hypertrophied cardiac muscle

$\beta$ -adrenergic pathway components	Behavior of the alterations
N of $\beta_1$ -adrenergic receptors /mRNA ( $\beta_1$ )	$\downarrow \Leftrightarrow / \downarrow \Leftrightarrow$
N of $\beta_2$ -adrenergic receptors /mRNA ( $\beta_2$ )	$\Leftrightarrow / \Leftrightarrow$
Gi protein activity/mRNA (Gi)	$\uparrow \Leftrightarrow / \Leftrightarrow$
Gs protein activity/mRNA (Gs)	$\downarrow \Leftrightarrow / \Leftrightarrow$
$\beta_1$ -adrenergic receptor activity	$\downarrow \Leftrightarrow$
Adenylate cyclase activity	$\downarrow$

N- number;  $\uparrow$  : increased;  $\downarrow$  : decreased;  $\Leftrightarrow$  : no alteration.

$\beta$ -adrenergic pathway in the genesis of functional alterations. Disagreements regarding the response to sympathomimetic stimulation, as well as the variability of anomalies of

the different components of the  $\beta$ -adrenergic pathway may result from the different types of experimental models used in the investigations.

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