

## The Concept of Crosstalk and its Implications for Cardiovascular Function and Disease

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At the beginning of a discussion on crosstalk among transduction routes in circulation, it is appropriate to seek a definition for this term. A more traditional definition for crosstalk would be the ability of different components of a given transduction pathway to influence components of another pathway. Many authors prefer a less restrictive definition that includes actions on common targets (“crossed activation”) or actions on separate targets, but that ultimately produce an identical effect on a single effector. Crosstalk is a growing concept as an underlying support for a coherently functional circulatory entity encompassing mechanisms that control the contractile apparatus, blood pressure, and circulating volume, both in normal physiology and in disease.

This review was planned as a form of helping students and cardiologists to better understand the dynamic concept and vital importance of crosstalk in cardiovascular function. Two important scientific texts were used as an organizational basis for this review, one from Saudi Arabia<sup>1</sup> and the other from the USA<sup>2</sup>. The former discusses the crosstalk of  $\beta$ 1-adrenoceptors. The latter, in reviewing cerebral circulation, focuses on concepts of endothelium and smooth vascular muscle functions from the viewpoint of crosstalk between the cGMP and cAMP systems.

According to the text by Dzimir, there are at least three signalling cascades directly associated with the physiological control of cardiac circulatory function:  $\beta$ 1-adrenoceptors that control the cardiac contractile apparatus; the renin-angiotensin-aldosterone system responsible for blood pressure control; and the natriuretic peptides that contribute with the factors that determine circulating blood volume<sup>1</sup>. Besides these pathways, other cardiac receptor systems, particularly the  $\alpha$ 1-adenosine, endothelin, and opioid receptors, whose physiological roles may not be readily evident, are also important for the control of cardiovascular function, especially in disease. These, and most of the other cardiovascular receptors identified to date, are coupled especially with three families of G-proteins: stimulatory (Gs), inhibitory (Gi) and Gq/11 proteins, stimulating

adenylate cyclase and phospholipases, activating a small but diverse subset of effectors and ion channels. When these pathways are linked to receptors, they are engaged in crosstalk that uses secondary messengers and protein kinases as intermediate mediators. Many signal transduction pathways contribute to the control of vascular smooth muscle tone. The activities of most of these pathways are perfectly controlled by a complex but “well-tuned” system, where the components of a given transduction cascade can interact with components of another pathway. This interaction was labeled crosstalk. Most experimental evidence that supports the idea of crosstalk between the cAMP and cGMP systems in smooth muscles was obtained in studies using peripheral vascular tissue.

### Crosstalk Cardiovascular Signalling

#### Crosstalk between subtypes of adrenergic receptors

The concept of a crosstalk receptor began in the early 1980s, when efforts were made to explain apparently incompatible behaviors of certain pharmacological agents. Consequently, among the descriptions of crosstalk made to date, the most elaborate is the one involving cardiac adrenergic receptors (AR), particularly subtypes  $\beta$ 1 and  $\alpha$ 1 that regulate contractility and heart rate<sup>3-7</sup>. This is partly attributable to the fact that, initially, the differentiation between the AR subtypes was hypothetically based on potency differences among the three agonists, epinephrine, norepinephrine, and isoproterenol for the  $\alpha$  and  $\beta$  subfamilies. Early studies had already demonstrated that a receptor classification had no basis because of the apparently paradoxical evidence that catecholamines not only provoke the transduction of their signals via  $\alpha$  and  $\beta$  ARs<sup>4</sup>, but also influence the components of individual signalling routes, virtually in the same way, but under different conditions<sup>5</sup>. These studies led to an evaluation of the probability of convergence of the two routes through adenylyl cyclase-G-protein-receptor signal transduction, and

### Key words

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this crossed regulation is carried out by the Gs and Gi proteins, regulating mechanisms that control cardiac contractile function under physiological conditions<sup>3,6</sup>.

### Crosstalk between adrenergic receptors and other cardiovascular receptors

The increase in specialized medical literature has quickly demonstrated that, apart from crossed regulation between the ARs, their stimulation activates changes in the transduction signal of other cardiovascular systems, particularly routes related to angiotensin receptors (ATR), endothelin receptors (ETR), muscarinic receptors of acetylcholine (MR), and receptors for natriuretic peptides and nitric oxide synthase (NOS). Growing evidence shows that the stimulation of these pathways can often increase or inhibit endogenous catecholamine release associated with the infra-regulation or desensitization of ARs. In the vascular system and peripheral circulatory organs, a complex crosstalk regulates ARs and is frequently associated with synergistic actions of various routes, or can be an indirect product of interactions among some non-adrenergic routes that act on the local release of catecholamines<sup>1</sup>.

### Crosstalk between non-adrenergic pathways

In addition to the interaction with ARs, the activation of several cardiovascular pathways that depend on signal transduction through the G-proteins, notably the angiotensin, endothelin, and muscarinic systems, can also activate the release of other vasoactive peptides such as the atrial natriuretic peptide (ANP), vasopressin, or aldosterone and help regulate cardiovascular responses.

The crosstalk between the vasoactive pathways happens on two distinct levels, the central nervous system, and cardiac humoral regulatory mechanisms. A classic example is the hypothalamic release of ANP through the crosstalk between MR and  $\alpha 1$  AR that are probably humorally regulated by the heart through several feedback mechanisms<sup>8</sup>. However, the actual mechanisms involved remain highly speculative.

The role of crosstalk in cardiovascular function would be incomplete without a brief consideration of its involvement in regulating ion canals of chloride (Cl<sup>-</sup>), sodium (Na<sup>+</sup>), potassium (K<sup>+</sup>), and calcium (Ca<sup>2+</sup>). These ion channels regulate the membrane potential and the transportation of ions and substrates, while they control excitation and excitation-contraction of the contractile apparatus. Regulation of these channels is often mediated by interaction between the pathways coupled with Gs and Gi proteins. For example, activation of the Na<sup>+</sup> pump and the potassium-dependent K<sup>+</sup> pump, mediates the hyper-polarization of vascular smooth muscle in relaxation induced by acetylcholine, possibly by activation of cGMP<sup>9</sup>.

### Crosstalk modulation between the cAMPc and cGMP systems

In order to facilitate the discussion of crosstalk between these systems, it is important to give a general description of the main pathways involved in the cyclic nucleotide cascades. For the cGMP system, focus will be on the cGMP generated

in vascular smooth muscle by soluble guanylate cyclase. Large-scale actions of cGMP are mediated by activation of cGMP-dependent protein kinase (PKG), which, in turn, regulates the function of target-proteins by phosphorylation. For cAMP, the receptor and G-protein enzyme associated with adenylate cyclase (AC), represent an initial step. As with cGMP, the functional effects of cAMP on vascular musculature are mediated primarily by kinase activation. However, cAMP not only promotes activation of the cAMP-dependent protein kinase (PKA), but can also activate PKG.

Based on a wider definition of crosstalk in the general structure commented above, the review by Pelligrino<sup>2</sup> was organized according to the following categories: 1) reciprocal effects of cyclic nucleotides on their corresponding levels. This would involve the capacity of a cyclic nucleotide to modulate the synthesis or degradation of the other. Any discussion in this area includes the capacity of the cAMP system, or of its components, to affect the synthesis of NO and cGMP and/or vice-versa (i.e. with cGMP affecting an activity of adenylate cyclase); 2) crossed activation of cyclic nucleotide kinases and/or superposition of its effects. This activation implies evidence that cAMP, within physiological limits, may activate PKA and PKG in vascular muscle tissue, and the possibility that both kinases can superpose their areas of influence (common sites of phosphorylation or "cooperative" phosphorylation); 3) intracellular compartmentalization of enzymes that mediate the synthesis of cyclic nucleotides, hydrolysis, and phosphorylation/dephosphorylation reactions related to cyclic nucleotides. This compartmentalization represents an additional level of crosstalk control and deserves attention. Nevertheless, it is important to emphasize that issues pertinent to cyclic nucleotide crosstalk presuppose that the transduction components reside in the same cell (i.e. smooth vascular muscle). Although this is frequently the case, there are many exceptions. In particular, systems related to cAMP are documented since they are more widely reported than systems related to cGMP<sup>10</sup>. At least seven (and possibly eight)<sup>11</sup> phosphodiesterase (PDE) isoforms have been identified so far (for a review, see Loughney and Ferguson, 1996)<sup>12</sup>. There are numerous examples of potentiation of cAMP-dependent vasodilatations by cGMP in peripheral vessels (for a review, see Komasa et al, 1996)<sup>13</sup>.

Another indirect pathway in which cGMP influences an adenylate cyclase activity that deserves consideration is related to the potential of NO to affect the synthesis of vasodilator prostanoids (see Dirosa et al, 1996, for a review)<sup>14</sup>.

The possibility that cAMP might act in the release of NO has been approached in several publications. Although a possible role for cAMP as a stimulant of endothelial NOS<sup>15-17</sup> or neuronal NOS<sup>18</sup> has been suggested, there is significant evidence that does not uphold this type of activity<sup>19,20</sup>.

### Implications of crosstalk between receptors in cardiovascular physiology

Cardiovascular signalling can be usually regulated at the level of one single functional entity, such as the contractile apparatus; however, more important is the possibility of coordinating different functions in one synchronized unit.

In executing these functions, two types of cellular responses, both short- and long-term, may develop. For example, short-term events include the activation of  $\text{Ca}^{2+}$  turnover in order to stimulate the contractile apparatus or vasoconstriction, whereas long-term actions are essentially involved in regulating the modified gene transcription or expression, often as an adaptive mechanism, such as left ventricular hypertrophy. Crosstalk-type cardiovascular signalling mediates both short- and long-term events and also coordinates individual reserve pathways in several functions. Even though, at large, most of the specific cardiovascular physiological signalling role has been clearly defined for most crosstalks among these transduction pathways, its existence in the cardiovascular system is strongly suggested as a functional role orchestrated in defense of classically defined pathways. Perhaps the most challenging question at present is how crosstalk is regulated beyond the receptor-G protein-second messenger circuit. Although this issue is far from answered, it may be plausible to assume that most of the "players" have been identified<sup>1</sup>.

The fact that heart function is regulated by several signalling cascades united by self-regulating and systemic regulating mechanisms obligates the heart to have an inherent machinery to integrate the communication among these individual pathways into one sole functional entity. To achieve this, the heart probably functions as an endocrine and paracrine organ, and determines its own destiny by regulating the several signalling mechanisms by crosstalk through receptors<sup>21,22</sup>. Some of these mechanisms originate in the central nervous system, and include: 1) possible blood pressure regulation in cardiovascular centers of the brain by acetylcholine (ACh) released by cholinergic neurons<sup>23</sup>; 2) the negative feedback system that regulates the equilibrium between vasodilation and vasoconstriction using mechanisms that involve crosstalk between ET-1, ET B, and NO<sup>24</sup>; 3) the regulation of noradrenergic and cholinergic systems in cardioinhibitor centers and vasomotor center in the oblongate medulla<sup>25</sup> and; 4) the regulation of muscarinic receptors by an  $\alpha$ -adrenergic system that controls ANP release by the hypothalamus<sup>8</sup>. As a result, the CNS can be intimately involved in the definition of the types, origins, and physiological entities which convert messages defined at the appropriate moment, using sympathetic and parasympathetic pathways between cardiac and extracardiac signals.

Regulation of the contractile apparatus through the crosstalk that signals engagement between crosstalk receptors with the three families of G-proteins, especially stimulatory (Gs), inhibitory (Gi), and Gq/11 proteins, uses phospholipase C (PLC) as a command center that regulates this crosstalk signalling. In positive inotropism regulation, for example, the  $\beta$ 1-Gs pathway is the main stimulator of the contractile apparatus. The vital role of crosstalk in regulating cardiovascular circulatory function is clear. Regulation of blood pressure and circulating blood volume is maintained by the crosstalk among several signalling routes, some of them controlled in the cardiovascular control centers of the central nervous system. Receptors such as AT1 and ET1 stimulate vasoconstriction using primarily PLC, which is also utilized by NOS and ANP in volemia regulation and smooth muscle cell relaxation. Finally, the potentially therapeutic objectives that

involve crosstalk as signalling in cardiovascular disease should be emphasized. Changes in cardiac disease progression to a terminal stage, such as cardiac hypertrophy, are frequently a result of long-term signalling effects<sup>1</sup>.

## Some Clinical and Therapeutic Implications

### Erectile dysfunction

Sildenafil revolutionized the treatment of organic erectile deficiency with its introduction in 1998. Not only is it effective, but it is an extremely acceptable way to treat erectile dysfunction (ED) (i.e., oral pharmacotherapy) when compared to other treatments available at that time (e.g., intracavernous injection). Sildenafil remains as the market leader for ED treatment, although some clinical efficacy failures have appeared in 'difficult- to-treat' groups of patients, such as diabetic, and side effects such as blue-tinted vision and headaches, besides the need to time the sildenafil dose for one hour before expected intercourse time. More recent phosphodiesterase inhibitors ("Viagra offspring") are available and potentially improve what was considered the golden standard of DE treatment. PDE5 is the predominant form of phosphodiesterase in the penile corpus cavernous where it degrades cGMP. Therefore, PDE5 inhibitors such as sildenafil, potentiate the endogenous increase in cGMP that is responsible for the nitric oxide-dependent vasodilation. This pharmacological treatment of erectile dysfunction is a classic example of crosstalk between the cAMP and cGMP systems. Its advent is perhaps the primary factor for the reappearance and reinforcement of the crosstalk concept<sup>26</sup>.

### Heart failure

In treating chronic heart failure, vasodilation agents, ACE inhibitors, and  $\beta$ -blockers increased life expectancy. Another strategy is an increase in myocardial inotropism utilizing phosphodiesterase inhibitors (PDEs), thus increasing intracellular cAMP, which increases intracellular calcium concentration and leads to a positive inotropic effect. An interesting assessment of the summarized data sought to review randomized data comparing the effects of PDEs to placebo in symptomatic patients with chronic heart failure. The key results, when compared to placebo, showed that treatment with PDEs correlated with a significant 17% rise in mortality rate. Considering all causes, the damaging effects of PDEs are consistent - with or without concomitant use of vasodilation agents - with the seriousness of the heart failure, and the PDE molecule or its derivative. These results confirm the fact that PDEs are responsible for an increased mortality rate compared to placebo in patients who suffer from chronic heart failure. Additionally, current results available do not support the hypothesis that the higher mortality rate is associated with additional vasodilator treatment. Consequently, the chronic use of PDEs should be avoided in heart failure patients<sup>27</sup>.

Muscarinic stimulation has an independent negative lusitropic effect and antagonizes the effects of  $\beta$ -adrenergic stimulation in the insufficient human left ventricle, whereas the blockage of muscarinic receptors has fewer effects on  $\beta$ -adrenergic responses in patients with heart failure. One

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potentially important implication of this fact is that the increased parasympathetic tonus in heart failure patients may have important effects on left ventricular function. This should be taken into consideration in future evaluations of parasympathomimetic agents for congestive heart failure treatment<sup>28</sup>. These data prove the presence of receptor crosstalk mechanisms in heart failure.

### Arterial hypertension and diabetes

Crosstalk between insulin and the sympathetic nervous system has possible implications in the pathogenesis of essential hypertension. Non-insulin dependent diabetic patients and obese patients have an elevated risk for developing arterial hypertension, while many non-obese, non-diabetic, and hypertensive patients have insulin-resistance in controlling induced glycemia, accompanied by hyperinsulinemia. This association has led some investigators to postulate that insulin resistance could be implicated in the pathogenesis of essential hypertension. Among the various factors considered as potential links between insulin-resistance and hypertension, the sympathetic nervous system can be considered a potential candidate. Recent findings clearly established that an acute activation of the sympathetic nervous system could antagonize glucose uptake mediated by insulin in skeletal muscle. The possibility of a primary defect in insulin sensitivity in hypertension being aggravated in the future by a greater sympathetic response evoked by episodic stimuli, such as postprandial hyperinsulinemia, becomes a reality. Nevertheless, while insulin evokes an increase in sympathetic nervous activity, at the same time it can conceal the vasoconstriction effects caused by reflex sympathetic activation. This modulating vascular effect is lost in essential hypertension, indicating that resistance to the effects of insulin in this disease is not only present in skeletal muscle metabolism, but also is evident at the vascular level<sup>29</sup>.

### Vasoplegic syndrome in heart surgery

Eversince the description of the vasoplegic syndrome in cardiac surgery by Gomes et al<sup>30</sup>, the systemic inflammatory response has attracted our interest for the last ten years. As is true in endotoxic shock, we presume that the inflammatory reaction is associated with an elevated endothelial release of NO because of NOS stimulation. Twelve years ago, we successfully used methylene blue in order to reestablish systemic arterial pressure and vascular resistance in a 72-year-old diabetic woman who experienced bradycardia and vasoplegic hypotension non-responsive to high-dose infusions of amines that began in the immediate post-operative period from myocardial revascularization surgery without complications. After this first bedside observation, Andrade et al reported a similar experience with six cardiac surgery patients operated with or without extracorporeal circulation<sup>31</sup>.

At present, the concepts discussed support the use of methylene blue as a reasonable treatment option for the vasoplegic syndrome, since it does not interfere in synthesis of NO, and since it is a widely used medication in other clinical conditions. Clinical experiences with methylene blue for treatment of vasoplegia associated with cardiac surgery

have been reported since the 1990s, in publications of letter/commentaries. Broader clinical investigations began to appear<sup>32</sup> and finally, two controlled and randomized studies were carried out which proved the prophylactic<sup>33</sup> and therapeutic<sup>34</sup> efficacy of methylene blue. A literature review demonstrates that methylene blue promotes blockage of cGMP by guanylate cyclase, and reestablishes hemodynamic parameters with a rapid restoration of catecholamine performances. These data are very suggestive of crosstalk between the cGMP and cAMP systems.

### Anaphylaxis

Considering NO as a final mediator of vasoplegia, Evora et al also used methylene blue to treat anaphylactic shock in eight patients, with a good immediate response<sup>35</sup>. Causes included the use of radiocontrast for urography, brain CT, and coronary angiography, besides one case related to the use of penicillin. Conventional treatment (adrenalin injections and corticosteroids) did not reverse the cardiocirculatory collapse, urticaria, and angioedema. These observations do not authorize the use of methylene blue as first choice for anaphylactic shock treatment. It is extremely important to emphasize that adrenalin continues to be the drug of choice. However, one can speculate on the synergism of drugs and on how this association stimulates the cAMP system and blocks the cGMP system, suggesting a crosstalk between both vasoregulatory systems.

### Pulmonary hypertension

Pulmonary hypertension (PH) can be of unknown etiology - primary pulmonary hypertension (PPH), or because of an underlying cause - secondary pulmonary hypertension (SPH). Pulmonary arteriolar vasoconstriction is considered an important characteristic of PH. In order to determine the clinical efficacy of sildenafil, a vasodilator that acts by PDE5 inhibition administered by any route to individuals with pulmonary hypertension in primary or secondary forms, electronic databases were consulted with a strategy previously determined by the Cochrane Foundation using current terms as of November, 2003. The vasodilation mechanisms of sildenafil, as in organic erectile dysfunction, include crosstalk between the cAMP and cGMP systems. The clinical use of sildenafil to treat PPH and SPH is growing. However, its true value, observed by a Cochrane-pattern meta-analysis, was not conclusive. The long-term effects based on NYHA functional class, symptoms, mortality, and exercise capacity still require additional confirmation. Further long-term studies, with adequate size and planning, are necessary in order to establish the true effects of sildenafil based on clinically significant results<sup>36</sup>.

### Summary and perspectives

This review could not be concluded without the important considerations made by Dzimiri.<sup>1</sup> The data reviewed clearly demonstrate the fact that the cardiovascular system is an extremely varied labyrinth, and includes several signalling pathways, firmly coordinated and perfectly synchronized to ensure harmony, integrity, and continuity of this vital function

for a lifetime. The most important question that remains is how the various pathways communicate with one another in order to carry out this noble function. Currently available data concur with the idea that notable cardiovascular transduction systems converge at certain checkpoints, probably under the humoral control of the heart itself. A malfunction or change in transduction of any one of these signalling routes can affect, positively or adversely, the signalling of another in regulating this function, and directs the near future research focus in this field. This summary is far from comprehensive, and merely represents a sample of the rapidly growing understanding on receptor crosstalk with potential relevance for the physiological regulation of the circulatory function. Interestingly, although this set of interactions among cardiovascular systems may seem congested and not very transparent, it is regulated merely by a coupling of G-proteins, protein kinases, and signalling junctions.

Finally, one word of caution is needed. The numerous

examples of crosstalk among transduction pathways reported in medical literature seem to insinuate a general common response of cells to different stimuli, even when these stimuli initially act on different cascades. This contradicts our knowledge of the specificity of action of extracellular signals in different cell types. This discrepancy is explained by the restricted occurrence of crosstalk in any type of cell and throughout various categories of specific cellular mechanisms. For example, the specific qualitative and quantitative expression of the various subtypes of transduction proteins, the combined control of cascades with specific steps of regulating factors, and the compartmentalization of the transduction cascades or of its elements. The subject of crosstalk is very complex, and, according to Dumont<sup>37</sup>, one very important question is pertinent: "Are we crosstalking ourselves into a general confusion?" At the end of this text we maintain this doubt, but we hope we have presented a review as short and objective as possible, in order to help undergraduate and graduate students, clinicians and even investigators.

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