Pathophysiological role of autoantibodies against G-protein-coupled receptors in the cardiovascular system

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After more than 15 years of intensive research in the field of functional autoantibodies (AAB) directed against G-protein-coupled receptors, there is growing evidence of a causal involvement of AAB in various cardiovascular diseases such as dilated cardiomyopathy, peripartum cardiomyopathy, malignant and essential hypertension, and preeclampsia. It has been indicated that AAB against beta-1 adrenergic receptor, alpha-1 adrenergic receptor, angiotensin-II receptor AT_1 and muscarinic M_2-receptors undergo agonist-like actions on the corresponding receptor and induce a permanent stimulation of G-protein-coupled signal cascades, which may cause Ca^{2+} overload and cardiomyocyte destruction.

Furthermore, the present review describes how G-protein-coupled receptor AAB are able to activate transcription factor nuclear factor-kappa B, which may regulate the expression of genes involved in immune and inflammatory responses.

Key Words: *Autoantibodies; G-protein-coupled receptors; Hypertension; Signal cascade*

Twenty-five years ago Venter et al (1) described the first autoantibodies (AAB) against beta-2-adrenergic receptors (β_2-ARs) in patients with allergic asthma. Later on, several groups (2-5) identified AAB against G-protein-coupled receptors (GPCRs). AAB against β_1/β_2-ARs and M_2-acethylcholine receptors were found in patients with Chagas' disease (6,7) and β_1-AR in dilated cardiomyopathy (DCM) (2). For identification of the AAB, Wallukat and Wollenberger (2) developed a sensitive bioassay consisting of spontaneously beating neonatal cardiomyocytes. Agonistic AAB, like AAB against the alpha $(\alpha)_1$-AR, β_1-AR or angiotensin-II receptor 1 (AT_1-R), cause an increase (positive chronotropy) of the beating rate. These AAB recognize the first or second extracellular loop of the receptors. Another group of AAB, which recognize the third extracellular loop, prevents the positive chronotropic effect caused by the corresponding agonists. These changes in beating rate can be documented by the bioassay used (2).

AAB AGAINST GPCR IN CARDIOVASCULAR DISEASES

Several cardiovascular diseases are associated with AAB directed against one or two GPCR (Table 1). AAB have been identified in the sera of patients with diagnosed heart diseases. Also, it has been hypothesized that a chronic myocarditis may develop into DCM (8). In both diseases, a virus infection and inflammation may be the primary etiological agent, and the inflammatory response may develop into an autoimmune disease (8). In patients with DCM, the prevalence of β_1-AR AAB can be up to 70% (3,4). In addition, a prevalence of 36% to 39% of muscarinic M_2-receptor AAB has been observed (5). These AAB undergo agonist-like actions on the receptor, but with effects contrasting those of the adrenergic system. The muscarinic M_2-receptor AAB induced, like the agonist carbachol, a negative chronotropic effect. Thus, there may be an overall balance in a subpopulation of patients with DCM (9).

AAB AND SIGNAL CASCADE

The GPCR are characterized by their seven-membrane spanning arrangement with three extra- and three intracellular loops. For epitope mapping, defined peptides of the extracellular domain of the receptors were used. These peptides, which neutralized the AAB-induced effects, characterized the epitopes on the receptor. For example, it was found that epitopes for α_1- and β_1-AR found in patients with essential hypertension (10,11) and DCM (3) are localized on the first or second extracellular loop, respectively (12). The epitopes of the AT_1-R AAB from preeclamptic women and patients with malignant hypertension are localized on the second extracellular loop (Figure 1) (13,14).

The binding of the AAB to the epitopes at the GPCR and the induction of the agonistic-like effects is not completely understood. For β_1-AR and AT_1-R, it has been shown that agonistic stimulation shifts the receptor to a dimeric state, stabilizes this confirmation and activates the signal cascade (15,16). We assumed that the agonistic AAB also realize their effects by a stabilization of this active dimeric conformation. In contrast to the physiologically regulated signal cascade, the AAB binding leads to a lack of receptor downregulation and a permanent over-stimulation of the GPCR (Figure 2). This results in an intracellular Ca^{2+} overload (as shown in the scheme of Freedman and Lefkowitz [17] for β_1-AR) with severe consequences for structure and function. The same over-stimulation was observed by activation of other GPCR AAB (eg, AT_1-R or α_1-AR) and of their specific signal cascade.

ACTIVATION OF NUCLEAR FACTOR-KAPPA B BY AAB

It is well known that several mediators (eg, cytokines, free radicals, bacterial or viral products such as lipopolysaccharides) rapidly activate nuclear factor-kappa B (NF-κB) by the phosphorylation and degradation of IκB. The phosphorylated

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TABLE 1
Autoantibodies directed against G-protein-coupled receptors in various diseases

Receptor type	Disease	Prevalence (%)
Alpha$_1$-R	Hypertension	44
β_1-R	Dilated cardiomyopathy	80
β_1-R	Myocarditis	80
β_1-R	Chagas' disease	29
β_2-R	Chagas' disease	12
β_2-R	Allergic asthma	ND
AT_1-R	Preeclampsia	80
AT_1-R	Malignant hypertension	14 to 33
AT_1-R	Vascular renal rejection	ND
Muscarinic M_2-R	Chagas' disease	77
Muscarinic M_2-R	Dilated cardiomyopathy	25
5HT4-R	Systemic lupus erythematosus	ND
Nicotinic AcCh-R	Myasthenia gravis	90
Glutamate receptor	Rasmussen's encephalitis, noninflammatory focal epilepsy, catastrophic epilepsy	ND
TSH-R	Grave's disease	>95

5HT4-R Serotonin receptor; β-R beta-Adrenergic receptor; AcCh-R Acetylcholine receptor; alpha-R alpha-Adrenergic receptor; AT_1-R Angiotensin-II receptor 1; M_2-R M_2-receptor; ND No data available; TSH Thyroid stimulating hormone

form of IκB dissociates from the NF-κB complex, and the NF-κB subunits, p50 and p65, are translocated from the cytoplasm into the nucleus. There they bind to cognate DNA sequences and, thus, regulate genes involved in both innate and adaptive immunity (18,19). Our aim was to see whether AAB against GPCR are also able to activate NF-κB. Using indirect immunofluorescence with an antibody against the 65 kD subunit of NF-κB, we recognized that α_1-AR AAB and AT_1-R AAB from patients with essential hypertension and preeclampsia activate NF-κB and cause the translocation of their subunits into the nuclei. Not only AAB, but also specific agonists (ie, phenylephrine instead of α_1-AR AAB, or angiotensin-II instead of AT_1-R AAB) induce activation and translocation. Control incubations with purified immunoglobulin G from healthy controls, and the pretreatment of the AAB with peptides corresponding to the extracellular loops of the α_1-AR or the AT_1-R prevented translocation of the NF-κB subunits.

CONCLUSIONS

After approximately 20 years of intensive research in the field of functional AAB directed against GPCR, there is evidence of a causal involvement of AAB in various diseases. Also, some cardiovascular disorders were identified as diseases in which AAB may play a pathogenic role. In the case of DCM, essential hypertension and preeclampsia, the autoimmune background adds a causal explanation to the well-known observation of GPCR over-stimulation by the adrenergic or renin-angiotensin system.

The present study supports the hypothesis that AAB against GPCR involve not only the short-term signalling cascade regulation of contractile processes, but also gene transcription by transcription factors like NF-κB or nuclear factor of activated T cells ($NFAT_1$) (20). This process may play an additional role in the pathogenesis of cardiovascular diseases.

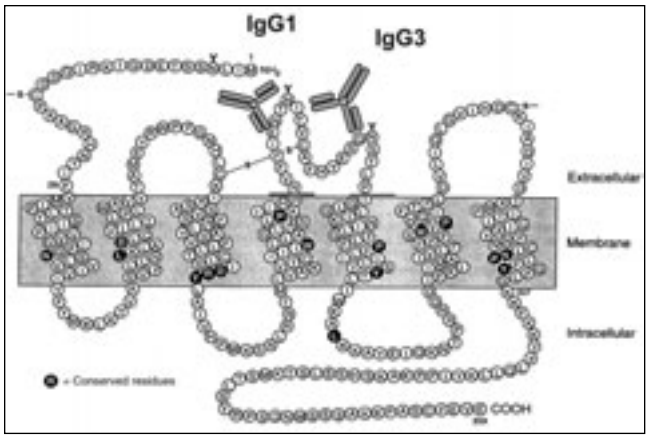

Figure 1) *Schematic structure of the human angiotensin-II type 1 receptor as a model of G-protein-coupled receptors. The angiotensin-II type 1 receptor autoantibodies of immunoglobulin G (IgG)1 and IgG3 bind on different amino acids of the second extracellular loop*

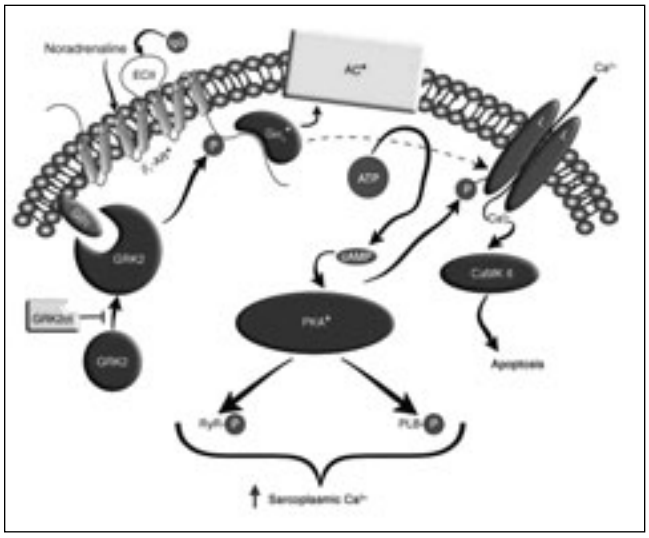

Figure 2) *Scheme of the signal cascade with beta $(\beta)_1$-adrenergic receptor (AR) autoantibodies according to Freedman and Lefkowitz (adapted with permission from reference 17). The seven-membrane-spanning receptor is stimulated by the physiological agonist noradrenaline or by the immunoglobulin G (IgG) of β_1-AR autoantibodies from patients with dilated cardiomyopathy or chronic heart failure. This stimulation activates the G-protein-adenylyl cyclase (AC) system with known consequences for augmentation of Ca^{2+}, activation of contractility and, finally, destruction of cardiomyocytes. CaMK II Calcium/calmodulin-dependent protein kinase II; cAMP Cyclic AMP; EC II Extracellular loop II; GRK2ct G-protein-coupled receptor kinase 2 C terminus; PKA Protein kinase A; PLB Phospholamban; RyR Ryanodine receptor*

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