

Regulation of Cardiac β_3 -Adrenergic Receptors in Hyperglycemia

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Beta-adrenoceptors (β -AR), members of the G protein-coupled receptors play important roles in the regulation of heart function. A positive inotropic action of catecholamines is mediated through their interaction with β -AR, located on the sarcolemma, while they can also mediate some deleterious effects, such as cardiac arrhythmias or myocardial apoptosis. The well-known β -AR-associated signaling in heart is composed of a coupled mechanism among both β_1 - and β_2 -AR and stimulatory G protein (G_s). This coupled mechanism further leads to the activation of adenylyl cyclase and thereby increases in intracellular cAMP level. However, recent studies have emphasized the contribution of constitutive β_3 -AR coupling to G_i proteins, thereby initiating additional signal transduction pathways, particularly under physiopathological conditions. Diabetic cardiomyopathy, as a distinct entity is recognized due to its diminished responsiveness to β_1 -AR agonist stimulation in the heart from diabetic rats with no important changes in the responses mediated with β_2 -AR. Furthermore, an upregulation of β_3 -AR has been shown in diabetic rat heart with a strong negative inotropic effect on left ventricular function. Experimental data provide evidences that the mechanisms for the negative inotropic effect with β_3 -AR activation appear to involve a pertussis toxin (PTX)-sensitive G protein and the activation of a nitric oxide synthase pathway. On the other hand, β -blockers demonstrate marked beneficial effects in heart dysfunction with scavenging free radicals and/or acting as an antioxidant with both sex- and dose-dependent manner. However, further investigations are needed to clarify the roles of both altered expression and/or responsiveness of β -AR and the benefits with β -blocker treatment in diabetes. This review discusses the role of β -AR activation, particularly β_3 -AR in cardiac pathological remodeling under hyperglycemia.

Keywords: β -Blockers, β -Adrenoceptor subtypes, Heart function, Oxidative stress, Diabetes mellitus.

Introduction

Cardiovascular dysfunction is one of the most important complications of chronic diabetes. In other words, diabetes is one of the major risk factors for the development of cardiovascular complications. A specific cardiomyopathy, independent from vascular dysfunction was first recognized by Rubler *et al.*¹ in diabetic patients with a marked mechanical dysfunction^{2,4}. Hyperglycemia is responsible for the diabetes-associated tissue damage detected in clinical events. This specific process is modified with several additional factors, such as genetic background of the individuals and independent accelerating factors, including environmental conditions and eating habits⁵⁻¹¹. Furthermore, several animal and human studies have demonstrated that neuroendocrine and/or metabolic responses to the similar stimulations might be different in a sex-dependent manner under

physiological conditions as well as pathological conditions, such as hyperglycemia¹²⁻¹⁴.

The importance of gender differences in incidence of cardiovascular dysfunction as well as morbidity and mortality has been reported. Studies performed under different pathological conditions have demonstrated the significant differences between women and men in terms of risk factors, symptoms and therapeutic responses^{14,20}. In addition, a marked depressed responsiveness to either inotropic or chronotropic β -AR stimulation is reported in cardiac contractile activity from diabetic individuals, which might be due to either a decrease in the density of cardiac β -AR, various subtypes of β -AR or both²¹⁻³¹.

The effects of hyperglycemia on cardiac β -AR subtypes are not known well, although some clinical and experimental data have shown the important contribution of β_3 -AR activation in cardiac dysfunction in diabetic individuals^{27,32-35}. However, it is not clear whether the changes in their activation and/or responsiveness to agonist stimulations are adaptive or not. It is well-accepted that hyperadrenergic state is an important marker for increased

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risk of mortality in patients with heart failure and, therefore, it is a logical to believe why treatment with β -blockers reduces the rate of mortality of these patients, as well as restores cardiac function in experimental animal studies^{19,20,36}.

The role of increased production of reactive oxygen species (ROS) in heart under hyperglycemia has been studied widely and discussed in many important review articles³⁷⁻⁴². It is well-known that hyperglycemia increases the production of ROS, alters the cellular redox status and causes rapid changes in membrane function, followed by contractile dysfunction within weeks in the diabetic heart⁴³⁻⁴⁸. The relation between β_3 -AR action and oxidative stress in cardiomyocytes under hyperglycemia is also reported in many animal studies^{27,33,49-55}.

This review has discussed particularly the role of β_3 -AR activation in diabetes-induced cardiac function and potential antioxidant-like therapeutic role of non-specific β -blockers in the diabetic subjects.

β -Adrenoceptor (β -AR) signaling in the heart under hyperglycemia

Hyperglycemia- and/or diabetes mellitus associated cardiac disorders seem to be common cause for morbidity and mortality among almost many countries, including underdeveloped countries. Catecholamines generally have important effects on the electrical activity and mechanical performance of the heart (via positive inotropic action), defined as due to activation of β -AR under physiological condition. It is demonstrated that excessive amounts of circulating catecholamines trigger changes in the β -AR system, leading to deterioration of ventricular function^{56,57}. This fact is accepted to be an adaptive mechanism of the heart to protect compromised myocardium from catecholamine overstimulation.

Much of the increase in cardiac output is due to the direct stimulation of β -AR in cardiomyocytes^{58,59}. The β -AR system belongs to G protein-coupled receptors (GPCRs), characterized by a conserved structural topology of 7-transmembrane domains with an extracellular N-terminus and an intracellular C-terminus. Activation of β -AR activates G_s and adenylyl cyclase (AC), resulting in the production of cAMP and subsequent activation of protein kinase A (PKA), which phosphorylates key components of the Ca^{2+} handling and contractile machinery⁶⁰.

Although sympathetic nervous system plays a central role in regulating heart function and response to pathologies through β -AR stimulation and there is evidence for impaired cardiac responsiveness to β -AR stimulation in experimental animals with diabetes; the results of these studies have not always been consistent^{22,25}. For instance, some investigators have reported a significant reduction in the number of myocardial β -AR in accordance with the diminished functional responses^{22,25,61}, while others have shown a reduction in β -AR sensitivity and the ratio of β -AR subtypes without altering receptor number⁵⁰.

Thus, it can be interpreted that exact nature of linkage between the functional depression in cardiac responses to catecholamines and variations in uncoupling of β -AR from the succeeding signal transducing systems in diabetes have not been clearly defined and needs further investigations.

Distinct β -adrenergic receptors subtype actions in diabetic heart

More than 40-years ago, Lands *et al.*⁶² recognized that β -AR could be classified into two distinct subtypes on the basis of their relative responsiveness for agonist stimulation. Early studies, both in human and animal models of diabetes have been undertaken only to determine abnormalities responsible for the depressed functional responsiveness to β -AR stimulation, without any consideration of their subtypes-dependent specified actions^{63,64}. It is known that cardiac β -AR system has three types — β_1 -AR, β_2 -AR and β_3 -AR and they differ significantly with respect to the types of cellular responses by their mediation^{65,66}.

Acute changes in cardiac function are controlled predominantly by β -AR signaling pathways. The signal transduction pathways triggered by agonist occupancy of β -AR are key regulators of the heart rate, systolic and diastolic function, as well as myocardial metabolism⁵⁷. Under hyperglycemia, chronic activation of sympathetic nervous system plays a major role in cardiac dysfunction via alterations of β -AR in their either expression, function or both. Interestingly, a depressed responsiveness related with either inotropic or chronotropic actions of β -AR agonist stimulation in cardiac contractility has been observed in experimentally-induced diabetes model animals^{24,67,68}. An impaired contractile response to β -AR agonist stimulation has been demonstrated in streptozotocin (STZ)-induced

diabetic rat heart with a 50% decrease in the density of β -AR, alteration in β -AR-G protein-AC system and phospholamban phosphorylation²⁵. A similar interpretation has also been presented by other studies^{22,69,70}.

In fact, very little is known about the molecular mechanisms of β -AR function in diabetic patients and animal models. A change in their total protein expression levels, the ratio of their subtypes and/or coupling among their subtypes are well-defined mechanisms underlying failing heart^{71,72}. Since cardiac dysfunction, mostly due to defects in Ca^{2+} handling system is predominant in diabetic subjects, a detailed review on the possible role of cardiac β -AR and their subtypes will provide further understanding of diabetic cardiomyopathy.

Recent studies have demonstrated that although β -AR signaling pathway plays important role in the regulation of heart function, the level of this signaling is controlled by the functional state, density and the subtypes ratio of these receptors^{27,50}. However, there are some contradictory studies related with the changes of β -AR subtypes and/or the total β -AR in left ventricular part of heart from diabetic individuals, such as either a decrease protein expression level of β_1 -AR, a decrease protein expression ratio of β_1 -AR to β_2 -AR, or a decrease protein expression ratio of β_1 -AR to β_2 -AR, together with an increase protein expression level of β_3 -AR^{22,25,27,50}.

Moreover, the functional data have also demonstrated that the positive inotropic effects due to β_1 - and β_2 -AR stimulations are decreased markedly in the isolated heart studies under hyperglycemia^{22,25,27,31,33}. Therefore, the mechanism(s) underlying the depressed cardiac responses to β -AR agonist stimulations is not clear yet, although to date, 3 types of β -AR have been cloned³¹⁻³³. On the other hand, evidence has been provided for the functional expression of β_3 -AR in the human and rat heart, its stimulation, in contrast to β_1 - or β_2 -AR, decreased contractile force^{33,35}. It has been also demonstrated that STZ-diabetic hearts exhibit decreased responsiveness to stimulation by β -AR agonists due to a decrease in β_1 -AR and an increase β_3 -AR expression^{26,27}. Therefore, the current studies strongly suggest that important role of β_3 -AR under pathological conditions may widely contribute to the controversies seen in the literature^{27,73-75}.

Role of β_3 -adrenergic receptors activation in diabetic cardiomyopathy

The effects of diabetes on cardiac β -AR responsiveness have been studied for many years

following the discovery mainly β_3 -AR subtype, besides β_1 - and β_2 -AR subtypes in the human heart⁷³⁻⁷⁵. In their study, Gauthier *et al.*⁷⁴ demonstrated a mediation of negative inotropic effect of β_3 -ARs stimulation by activation of a nitric oxide synthase (NOS) pathway in human ventricle. Furthermore, in another study, an upregulated β_3 -AR expression with a negative inotropic effect on cardiac contractility has been also demonstrated in cardiac ventricle from diabetic rats²⁷. The negative inotropy via β_3 -AR activation involves the inhibitory G_i protein^{74,76}, and the production of NO by NOS leads to an increase in intracellular cGMP level⁷⁷. Although the exact mechanism underlying the functional loss of catecholamine positive control of cardiac contractility is not clear yet, the current data provide information on a significant shift from β_1 -AR mediated positive inotropic effect to β_3 -AR mediated negative inotropic effect^{76,78}.

The effects of hyperglycemia on β -AR expression, function and downstream signaling seem to be controversial, in most, due to animal model, diabetes period and cardiac preparation^{25,27,50,79}. In addition, we have observed a sex-dependent β_1 -AR responsiveness of cardiac contractility from 4-weeks diabetic rats to a different extent, such as a sex-dependent cAMP responses via β_1 -AR with a similar β_2 -AR responses in both sexes¹⁶. On the other hand, a blunted chronotropic response to noradrenaline has been observed in 14-weeks, but not 8-weeks diabetes, while the response to a selective β_2 -AR agonist fenoterol is preserved²⁶. These studies strongly suggest that protein level of β_1 -AR is markedly decreased with a significant negative inotropic effect in the heart under hyperglycemia, while the β_3 -AR level is increased, being parallel to its negative inotropic action on cardiac contractility^{16,27}.

In our study, we examined the protein expression levels of total β -AR, β_1 -AR, β_2 -AR and β_3 -AR in isolated cardiomyocytes from left ventricles of 12-weeks STZ-diabetic male rats. As can be seen from Fig. 1A, the protein level of β_3 -AR is increased 2-fold with respect to the aged-matched controls, although protein level of total β -AR is found to be not different among the diabetic group and the aged-matched control group⁸⁰. In addition, the β_3 -AR agonist (BRL37344) responses of left ventricular developed pressure (LVDP) in diabetic group is depressed, compared to those of the controls (Fig. 1B) which is also in line with previously published data^{52,76}.

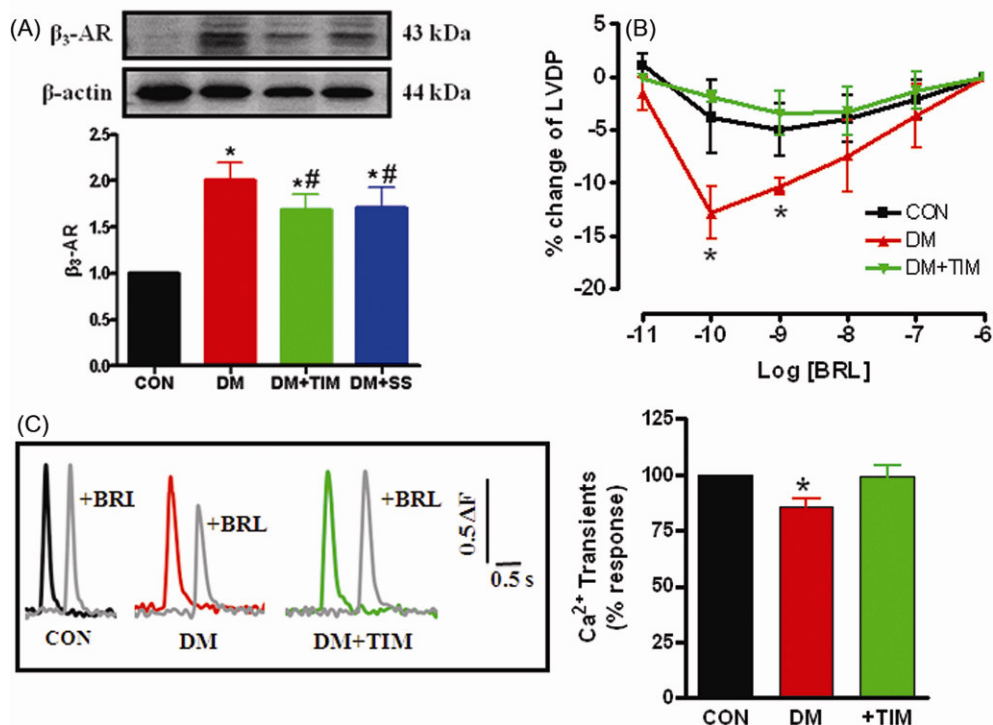


Fig. 1—Effect of diabetes on expression level and function of β_3 -AR in myocardium [(A) Upper part: Representative Western blots of β_3 -ARs measured in heart homogenates from control (CON), diabetic (DM), timolol-treated (DM + TIM) and sodium selenate-treated (DM + SS) rats. Lower part: Quantification for the ratio of β_3 -AR to β -actin in the above groups, respectively. Bars represent mean \pm SEM, $n = 5-6$ for hearts/group/protocol (double assays in each sample from each group for each type of measurement). Significant at * $p < 0.05$ vs. CON and # $p < 0.05$ vs. DM; (B) Effects of submaximal concentrations of a specific β_3 -AR agonist, BRL37344 on left ventricular developed pressure, LVDP in treated and untreated diabetic groups, compared to that of the control group. Here, the concentration–response curves for BRL37344 represent the inotropic responses as % of their initial values. Significant at * $p < 0.05$ vs. CON; and (C) Representative Ca^{2+} transients in freshly isolated cardiomyocytes loaded with Fura-2 and field-stimulated at 0.2 Hz (left) and the mean changes in peak amplitude of the fluorescence related with global Ca^{2+} transients ($\Delta F_{340/380} = F_{340/380} \text{ Peak} - F_{340/380} \text{ Basal}$) (right). Bar graphs represent mean \pm SEM of 12-17 cells from at least 5 animals for each group protocol. Significant at * $p < 0.05$ vs. CON and † $p < 0.05$ vs. DM by using one-tail student t-test]

Indeed, it has been previously shown that stimulation of cardiac β_3 -AR, in contrast to β_1 -AR and β_2 -AR stimulations induces a marked decrease in contractile force of human ventricular strips^{74,81}. The possible underlying mechanisms related with β_3 -AR stimulation-mediated negative inotropic effect in cardiac contractility may include an involvement of G_i and NO-cGMP-PKG in the β_3 -AR signaling pathway^{74,82-84}. In addition, although various studies provide functional evidence for the role of β_3 -AR modulation of ventricular function in animal models^{26,27,74,80}, it remains to be established whether the effects of β_3 -AR stimulation on cardiac function, particularly under pathophysiological conditions are beneficial or detrimental.

Furthermore, a clear explanation for these controversies is provided in some studies on the role of β_3 -AR stimulation in the cellular level. For

example, negative inotropic effect of β_3 -AR stimulation has been shown to be associated with alterations of action potential parameters⁷⁴ and decreased transient intracellular Ca^{2+} changes under electrical stimulation in part via a NOS pathway⁸⁵. Moreover, it is also demonstrated that a selective β_3 -AR agonist BRL37344 inhibits L-type Ca^{2+} -channels and Ca^{2+} -transients in canine ventricular cardiomyocytes, which are partly abolished by L-NAME⁸⁶⁻⁸⁸. We have performed similar experiments in isolated ventricular cardiomyocytes loaded with Fura-2 from diabetic rats under electrical stimulation and measured transient intracellular Ca^{2+} changes. As can be seen from Fig. 1C, response to a β_3 -AR agonist BRL37344 in the diabetic group is significantly less, compared to that of the control, of which is further in line with increased protein level of β_3 -AR. In these diabetic

animals, we have previously demonstrated that the L-type Ca^{2+} -currents measured in the ventricular cardiomyocytes are significantly inhibited, compared to those of the age-matched controls⁸⁰.

Our previous and the present data are further supported by the study of Zhang *et al.*⁸⁸, showing an enhanced inhibition of L-type Ca^{2+} -current by β_3 -AR stimulation in failing rat heart. In their study, since the inhibitory effect of BRL37344 is attenuated by a NOS inhibitor, L-NAME and is prevented by the incubation of myocytes with PTX, they have concluded that β_3 -AR activation could inhibit the L-type Ca^{2+} channel in both normal and heart failure myocytes, due to a coupling with PTX-sensitive G-protein and partially mediation through a NOS-dependent pathway.

Cardioprotective effect of β_3 -adrenergic receptors antagonism with β -blockers in diabetes

The various studies provide strong evidence that β_3 -AR stimulation plays important roles in the remodeling of cardiovascular function in heart failure under several pathological conditions including diabetes^{25,27,50,52,76,79}. Thus, it can be concluded that chronic cardiac dysfunction is associated with diminished β_1 -AR, but enhanced β_3 -AR function which are associated with the changes in their protein expressions levels, as mentioned previously in the above section. Whether this process is a protective response to catecholamine over-expression or a contributor to heart dysfunction has been controversial yet. However, clinical and experimental studies suggest using β -blockers as new therapeutics not only in diabetes, but also in other types of heart failure status^{33,83,89}.

Earlier studies have shown that patients with heart failure have an increased hyper-adrenergic state and, therefore, their treatment with β -blockers reduces the rate of mortality of these patients, as well as restoring cardiac function in experimental animal studies^{36,90}. Several studies have investigated the preventive role of various β -blockers on the development of heart failure by restoring cardiac Ca^{2+} release channels, ryanodine receptor (RyR2) dysfunction^{19,20,47,80,91,92}. Furthermore, it is reported that non-selective β -blockers, apart from their β -blockage action, exert adrenoceptor-independent effects, including scavenging of free radicals, leading to well-controlled cellular redox status^{93,94}. We have also reported that 3-months non-selective β -blockers treatment with either timolol or propranolol has beneficial

effect on heart function in male rats during increasing age, whereas only timolol, but not propranolol exerts similar beneficial effects in female rats^{19,20,80}.

The effects of timolol seem to be related with its cardio-preventive action via regulation of intracellular Ca^{2+} signaling and left ventricular remodeling, in part, due to its antioxidant action, which includes both its systemic action and direct action on heart tissue in the diabetic rats⁸⁰. Furthermore, in a cell culture study, both nipradilol and timolol are potent protective agents against increased oxidative stress⁹⁵. A direct ROS scavenging action of timolol is also shown, comparison with other β -blockers using *in vitro* studies with different cell lines^{95,96}.

Moreover, we have also shown previously a significant cardioprotection with timolol in a female rat model of aging-related altered left ventricular function via prevention of the antioxidant system dysfunction, including increased lipid peroxidation, decreased ratio of reduced glutathione to oxidized glutathione and decreased activities of thioredoxin reductase and glucose-6-phosphate dehydrogenase of tissue homogenates from left ventricle of the heart^{19,20}. Thus, it is necessary to investigate whether or not timolol action is different from the other known β -blockers in diabetic subjects, due to its dual properties as an antioxidant and blockade of signal transduction.

We have examined the protective effect of timolol treatment (5 mg/kg daily for 12 weeks) on the expression and function of β_3 -AR stimulation in Langendorff-perfused isolated heart from diabetic rats. As can be seen from Fig. 1A to C, timolol treatment has important protective action in cardiac dysfunction in diabetic rats via affecting stimulated β_3 -AR at both organ and cellular levels. Using biochemical data from circulatory system and the heart of diabetic and timolol-treated diabetic rats, we have demonstrated that protective action of timolol, directly targeting heart, seems to be associated with its controlling the cellular redox status in hyperglycemic cardiomyocytes⁸⁰. For comparison, we have examined the expression level of β_3 -AR in the heart preparation from an antioxidant, sodium selenate (0.3 mg/kg daily for 4 weeks) treated diabetic rats. As can be seen from Fig. 1A, the prevention of diabetes-induced upregulation level in the β_3 -AR expression is similar. Therefore, increased oxidative

stress in the heart under hyperglycemia plays important role in cardiac dysfunction via contribution of upregulated β_3 -AR.

Although we and others have reported important benefits with β_3 -AR blockade in cardiac studies from timolol-treated diabetic rats, Sharma *et al.*⁵² have shown a beneficial role of metoprolol treatment on cardiac function in diabetic rats via increasing of β_3 -AR expression level and downstream Akt-mediated signaling. Similar beneficial effects due to β_3 -AR activation have been also reported in different animal model studies⁹⁷⁻⁹⁹.

Concluding remarks

Diabetes is one of the important metabolic disorders, where incidence and prevalence are increasing rapidly. Indeed, it is well-accepted that development of cardiovascular complications due to diabetes is one of the major effects on humans^{37,39}. In addition, although there are some contradictions between the experimental results in the literature, the known data suggest the important contribution of increased oxidative stress, induced by not only ROS, but also reactive nitrogen species (RNS) derived via hyperglycemia, directly and/or indirectly to the structural and functional damages in the diabetic cardiac tissue^{39,42}. Furthermore, altered levels of β -AR subtypes in diabetes-induced cardiac dysfunction have been suggested as another important contributing signaling pathway in various studies^{33,83,89,98,100,101}. This hypothesis is strongly supported by the studies on treatments with different β -blockers in animal models, which depends on their adrenoceptor-independent effects, including scavenging of free radicals via leading to controlled cellular redox status^{93,94}. In addition, recently, we have also shown that timolol treatment (for 12-weeks) prevents diabetes-induced depressed left ventricular basal contractile activity, prolongs cellular electrical activity and attenuates the increase in cardiomyocyte-size without any anti-hyperglycemic effect. Our Western blot analysis has demonstrated that timolol treatment also significantly normalizes depressed levels of some intracellular Ca^{2+} -handling regulators, such as $\text{Na}^+/\text{Ca}^{2+}$ exchanger and phospholamban. Interestingly, our *in vitro* experiments have shown that timolol also leads to a balanced oxidant/antioxidant level in both heart and circulation and prevents altered cellular redox state of the heart.

To better understand the mechanism underlying the modification of β_3 -AR expression and function in

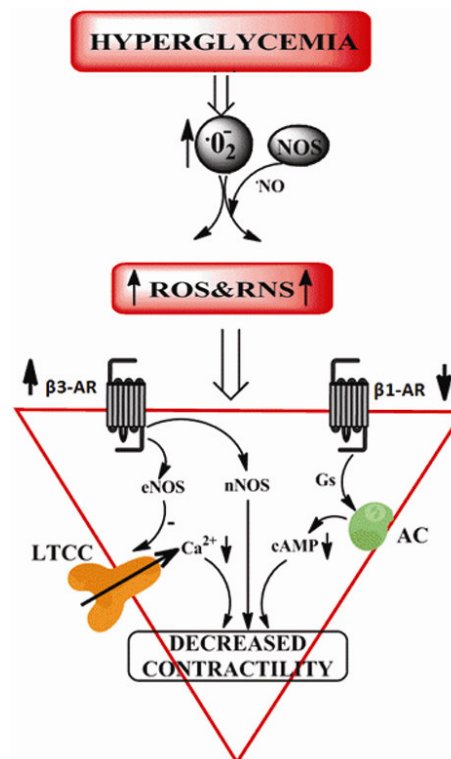


Fig. 2—Proposed mechanism representing our hypothesis on the roles of stimulated β_3 -AR and inhibited β_1 -AR in hyperglycemia-induced depressed cardiac contractility [LTCC: L-type Ca^{2+} -channels, NOS: nitric oxide synthase, eNOS & nNOS: endothelial and neuronal NOS, AC: adenylyl cyclase, G_s: stimulatory α -subunits of G-proteins; ROS: reactive oxygen species, RNS: reactive nitrogen species]

diabetic heart and its relationship with increased oxidative stress, we propose here a hypothetical pathway (Fig. 2), based on our previously published and the present data. In diabetic heart dysfunction, β_1 -AR is downregulated or desensitized, via G_s and AC signaling, leading to less cAMP production and less contractility. On the other hand, it can be suggested that hyperglycemia may induce upregulation in both β_3 -AR and nNOS levels via a cross-talk between G_i and eNOS. These events seem to be correlated with a downregulation in eNOS, which further inhibits the L-type Ca^{2+} -channels. Consequently, this cross-talk or a direct nNOS upregulation further can induce a marked depression in contractile activity of the heart under hyperglycemia.

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