Diabetes decreases mRNA levels of calcium-release channels in human atrial appendage

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Abstract

Patients with chronic diabetes mellitus usually develop reductions in rate and force of cardiac contractions. Since calcium-release channels (ryanodine receptors (RyRs) and inositol 1,4,5-trisphosphate receptors (IP3Rs)) play integral roles in effecting these processes, we rationalize that alterations in their expression may underlie these defects. To test this hypothesis, right atrial appendages were obtained from diabetic (65.0 ± 4.5 years) and nondiabetic (56.2 ± 2.6 years) patients undergoing coronary arterial by-pass grafting and reverse transcription-polymerase chain reactions were used to compare steady state levels of mRNA encoding the three major isoforms of RyRs and IP3Rs. In this study we did not detect either RyR1 or RyR3 in human atrial appendage. When compared with nondiabetic patients, mRNA encoding RyR2 from diabetic patients decreased by 74.2 ± 6.2% (p < 0.01). Diabetes also significantly decreased steady-state levels of mRNA encoding the IP3Rs in human atrial appendage. IP3R1 decreased by 24.2 ± 4.6%, IP3R2 decreased by 63.0 ± 4.6% and IP3R3 decreased by 55.5 ± 6.5%. Since a reduction in steady-state mRNA is usually indicative of a decrease in protein levels, these data suggest that the decrease in chronotropy and inotropy seen in chronic diabetic patients may be due in part to a decrease in expression of calcium-release channels. (Mol Cell Biochem 263: 143–150, 2004)

Key words: calcium-release channels, diabetes, expression, human atrial appendage, inositol, 1,4,5-trisphosphate receptors, reverse transcription-polymerase chain reaction, ryanodine receptors

Introduction

Individual with diabetes mellitus (types 1 and 2) usually develop cardiovascular complications independent of coronary arteriosclerosis, nephropathy, and micro/macraangiopathy [1–3]. This diabetic cardiomyopathy which significantly reduces overall cardiac performance is manifested clinically as reductions in heart rate, left ventricular systolic pressure development, peak tension and relaxation kinetics [4–7]. While the mechanism(s) underlying each of these functional changes may be different, a common feature in experimental models of diabetes used to study this syndrome is an alteration in expression and/or function of several key proteins involved in regulating/maintaining intracellular ionic homeostasis [8–10]. One such group of proteins is the intracellular calcium-release channels, commonly referred to ryanodine receptors (RyRs) and inositol 1,4,5-trisphosphate receptors (IP3Rs) [11–15]. To date, three distinct isoforms of each of these receptor types have been cloned and characterized and are referred to as RyR1, RyR2, RyR3, IP3R1,
IP$_3$R2 and IP$_3$R3, respectively [16–19]. Though structurally related, these two channel types are functionally distinct and are coupled differently to plasma membrane proteins (involved in distinct signal transduction cascades). It is generally accepted that the activity of IP$_3$Rs is modulated by G-protein coupled membrane-bound receptors, while calcium ions are mobilized from the sarcoplasmic reticulum (SR) via RyRs when the cells are electrically depolarized.

In the heart, RyR2 is the major release channel through which calcium ions leave the SR to effect rhythmic contractions [20]. This receptor is found in both atrial and ventricular tissues and is located predominantly in triad junctions, although some are also found on non-junctional or corbular SR [21, 22]. More recently, RyR1 and RyR3 have been also detected throughout the human heart [23]. However, at this time the physiological role(s) of the latter isoforms remains undefined.

IP$_3$Rs are also present in both ventricular as well as atrial tissues [24]. However, their functional roles may be more diverse. Studies have suggested that these receptors are mobilized following activation of adrenergic receptors (both alpha and beta), sigma receptor and endothelin receptors [25–27]. Tissue localization may also suggest specialize functions. Fleischer and co-workers [28] showed that these receptors are located to intercalated discs suggesting involvement in cell–cell communication. Some of these functions may also be isoform-selective. For example, Gorza et al. [29] showed that IP$_3$R1 are present in high levels on Purkinje cells, while Perez et al. [30] showed that working myocytes from rat and ferret ventricular tissues also express high levels of IP$_3$R2. As to why distinct isoforms of IP$_3$Rs are employed for these different functions remain to be delineated. However, what is known is that the affinities of endogenously generated IP$_3$ (ligand for the receptor) vary among IP$_3$R isoforms (IP$_3$R2 ≫ IP$_3$R1 ≫ IP$_3$R3). It should also be mentioned that although the activity of RyRs and IP$_3$Rs are differentially regulated, studies have shown that a significant amount of cross-talk also occurs between these two receptor classes [31]. That is, calcium release from the SR via IP$_3$Rs (smaller amounts) can serve to activate RyRs, triggering them to release of larger amounts of calcium ions.

Using STZ-diabetic rat model, we and others have shown the expression of RyR2 decreases in hearts of long-term diabetic rats [11–14]. More recently Razeghi et al. [8] showed that expression of RyR2 decreases in left ventricular apex from chronic diabetic patients. What remains to be ascertained at this time is whether expression of this receptor subtype as well as other calcium-release channels is altered in other regions of the heart from diabetic patients. In this study, the effect of chronic diabetes on steady state mRNA levels of calcium-release channels was investigated in right atrial appendage from diabetic patients.

Material and methods

Patient characteristics

Protocols for collection, storage and analysis of human tissues were reviewed and approved by the Baskent University School of Medicine and University of Ankara Ethics Committees. During a 2-month period, 51 right atrial appendages were obtained from diabetic and non-diabetic patients with sudden angina pectoris undergoing coronary by-pass surgery at Baskent University, Ankara Turkey. Age, sex and medical history of these patients were obtained post-surgery and the following criteria were used for selection of sample set for this study; (i) patient (non-diabetic and diabetic) did not suffer from any prior acute myocardial infarction and/or heart failure, (ii) patient (non-diabetic and diabetic) was angiographically shown to have coronary artery diseases, differences between non-diabetic and diabetic tissues most likely reflect the presence of diabetes, not just due to the consequences of ischemia (iii) non-diabetic patient did not have history of cardiac diseases, and (iv) diabetic patient had been diagnosed with the syndrome for at least 5 years and has been receiving insulin-therapy for at least 2 years. Lifestyle (e.g., diet, cigarette smoking, alcohol consumption, etc.) of patients was not a selection criterion for this study. Five diabetic and five non-diabetic samples that satisfied these criteria were selected for analysis. All diabetic patients selected (age, 65 ± 4.5 years; sex, 4F/1M) were on insulin therapy (24 ± 5 U per day) and aspirin. In addition, two were on a calcium channel antagonist and another two were on nitrovasodilator therapy. Non-diabetic patients (age, 56.2 ± 2.6 years; sex, 4M/1F) were either on calcium channel antagonists (n = 2), ACE inhibitors (n = 2) or aspirin (n = 2) therapy. None of the patients selected for this study received beta-adrenergic receptor blocker as part of the therapeutic regimen prior to surgery. Dolantin, promethazine and atropine were given as pre-medications and all surgical procedures were carried out under balanced fentanyl and isoflurane anaesthesia. Heparin, prednisolone, dopamine, nitroglycerin and anti-arrhythmics were also administered to some patients. Blood glucose levels of all diabetic patients were normalized (120–150 mg/dL) before surgery.

Isolation and quantitation of total RNA

During surgery, right atrial appendage (∼100 mg tissues) was removed from patient and placed in liquid nitrogen. The frozen tissue was then removed, labelled and stored at −80 °C. Total RNA was extracted simultaneously but separately from diabetic and non-diabetic samples using Quick Prep® total RNA extraction kit (Amersham Pharmacia Biotech Inc., Uppsala, Sweden). A sample of total RNA (10 µg) was run on an agarose gel. The RNA band was excised from the gel and purified by electroelution. Total RNA samples were then treated with DNase I (Ambion, Austin, TX) before reverse transcription. A total of 0.5 µg of total RNA was reverse transcribed in a final volume of 20 µl for each sample using a first-strand cDNA synthesis kit (Applied Biosystems, Foster City, CA).