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# Decrease in calcium-sensing receptor in the progress of diabetic cardiomyopathy

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ABSTRACT

To observe the dynamic expression of calcium-sensing receptor (CaSR) in myocardium of diabetic rats and explore its role in diabetic cardiomyopathy (DCM), 40 male Wistar rats were randomly divided into 4 groups including control, diabetic-4 weeks, diabetic-8 weeks and spermine treatment groups (240 μM of spermine in drinking water). The type 2 Diabetes mellitus (DM) models were established by intraperitoneal injection of streptozotocin (STZ, 30 mg/kg) after high-fat and high-sugar diet for one month. The echocardiographic parameters were measured, cardiac morphology was observed by electron microscope and HE staining. The intracellular calcium concentration ($[Ca^{2+}]_i$) was detected by laser-scanning confocal microscope. Western blot analyzed the expression of CaSR, protein kinase C α(PKC-α) and calcium handling regulators, such as phospholamban (PLN), $Ca^{2+}$-ATPase (SERCA), and ryanodine receptor (RyR). Compared with control group, $[Ca^{2+}]_i$ and the expression of CaSR, RyR and SERCA/PLN were decreased, while PKC-α and PLN were significantly increased in a time-dependent manner in diabetic groups. Meanwhile diabetic rats displayed abnormal cardiac structure and systolic and diastolic dysfunction, and spermine (CaSR agonist) could prevent or slow its progression. These results indicate that the CaSR expression of myocardium is reduced in the progress of DCM, and its potential mechanism is related to the impaired intracellular calcium homeostasis.



## 1. Introduction

Diabetes mellitus (DM) is characterized by elevated blood glucose levels resulting from the body's inability to produce insulin or resistance to insulin action, or both [1]. The most prevalent form (90–95%) of DM is type 2 diabetes (non-insulin-dependent) [2]. Diabetic cardiomyopathy (DCM), a serious DM complication, can cause heart failure, arrhythmia, cardiac shock and sudden death [3]. However, the underlying molecular mechanisms on DCM largely remain unclear [4].

The extracellular calcium-sensing receptor (CaSR) is a member of the G protein-coupled receptors superfamily, whose primary function is to regulate parathyroid hormone

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secretion and renal $Ca^{2+}$ reabsorption, thus it is crucial to the control of extracellular $Ca^{2+}$ homeostasis [5]. In 2003, our team first found that CaSR exist functionally in rat cardiac tissue [6], and sequentially demonstrated that CaSRs are involved in cardiac ischemia/reperfusion injury, apoptosis, calcium overload, endoplasmic reticulum stress, ischemic post-conditioning, cardiac hypertrophy and artherosclerosis, etc. [7–10]. However, the potential role of CaSR in the progress of DCM has not been examined.

The activation of CaSR can cause intracellular $Ca^{2+}$ increase by inducing calcium release from sarcoplasmic reticulum (SR) and opening of storage operated calcium channel (SOCC).

In addition, other players in storage operated calcium entry/calcium release activated calcium currents such as STIM1/Orai1 in the myocardium [11].

With this background, the aim of this study is to observe changes in cardiac CaSR expression in type 2 diabetic rats, and will discuss potential mechanisms.

## 2. Materials and methods

### 2.1. Establishment of type 2 diabetes rats

Forty male Wistar rats, 180–200 g, were provided by the Experimental Animal Center of Harbin Medical University. Type 2 diabetic models were prepared as previously described [12]. All rats were housed in a temperature-controlled room (22–24 °C) and kept on a 12-h:12-h light–dark cycles in quiet environment. All rats received humane care in accordance with the principles of the Chinese Council on Animal Care. After one week's adaptation, the rats were randomly divided into control, diabetic-4 weeks (dia-4w), diabetic-8 weeks (dia-8w) and spermine treatment (dia + sper) groups. Control rats were maintained on standard rat chow and tap water ad libitum. Diabetic rats were fed with high-fat and high-sugar chow (ingredients: 20% sucrose, 10% refined lard, 2% cholesterol, 1% sodiumcholate and 67% common food). One month later, experimental rats were induced by an intraperitoneal injection of a low dose of streptozotocin (STZ, Biosharp, Japan, 30 mg/kg), dissolved in 0.1 M citrate buffer (pH 4.4). The blood glucose level was detected after 3 days, only the rats with blood glucose level $\geq$16.7 mM were considered to be diabetic and used in our experiments. Age-matched control rats were injected with a vehicle alone. The diabetic rats maintained on high-fat and high-sugar and tap water ad libitum for 4 or 8 weeks. The rats in dia + sper group received 240 μM of spermine (lower than toxic dose) [13] in their drinking water for 4 weeks. Blood glucose, food and water intake levels were determined on a weekly basis. After the end of experiment, all animals were sacrificed and their hearts were stored at −80 °C for further studies.

### 2.2. Serum measurements

The blood samples were centrifuged and serums were stored at −80 °C until assay. Random serum insulin levels were determined by a commercially available ultrasensitive ELISA kit (Morinage Institute of Biological Science Inc., Kanagawa, Japan). Triacylglycerol (TG) and cholesterol (CHE) in serum were analyzed using a standard biochemistry panel (Senlo, Zhuhai, Guangdong). Random blood glucose in blood samples from tail vein was measured using a blood glucose analyzer (ACCU CHEK, Roche, Germany).

### 2.3. Echocardiographic analysis of cardiac structure and function

Cardiac function and dimensions were assessed using an echocardiography system (GE VIVID7 10S, USA). Echocardiography was performed on self-breathing rats under anesthesia (intraperitoneal injection of 10% chloral hydrate at 0.3 ml/100 g body weight) as described previously [14]. Heart rate was kept relatively constant throughout the procedure. The following left ventricular (LV) parameters were measured: left ventricle end-systolic lumen diameter (ESLD), fractional shortening (FS), ejection fraction (EF), the early transmitral peak diastolic flow velocity (*E*), peak early diastolic tissue velocity (*E*′). The ratio of *E* to *E*′ (*E*/*E*′ ratio) was calculated for analyzing cardiac diastolic function. All parameters represent the mean of 3 consecutive cardiac cycles.

### 2.4. Morphologic study

After echocardiography assessment, the rats were anesthetized, hearts were rapidly removed and washed with phosphate buffered saline solution. The cardiac tissues fixed in 10% buffered formalin were embedded in paraffin, sectioned at 4 μm and stained with hematoxylin and eosin (HE) for light microscopic morphologic study.

### 2.5. Transmission electron microscopy

For transmission electron microscopy, rat left ventricular tissue was fixed in 2.5% glutaraldehyde, cut into small blocks (1 $mm^3$), and fixed at 4 °C for 8 h. Images of the longitudinal sections were obtained after fixation, soaking, stepwise alcohol dehydration, displacement, embedding, polymerization, sectioning, and staining and observed with an electron microscope (H-7650, Japan). Random sections were taken and analyzed by two technicians blinded to the treatments.

### 2.6. Isolation of cardiomyocytes from diabetic rats

Ventricular myocytes were isolated from normal and diabetic rats according to previously described techniques [15]. Hearts were removed rapidly after anesthetization, connected to the Langendorff apparatus and perfused at a constant flow and at 37 °C with a Tyrode's solution containing (mM): 140 NaCl, 5.4 KCl, 0.6 $MgCl_2$, 0.6 $Na_2HPO_4$, 1 $NaHCO_3$, 10 HEPES, 1.8 $CaCl_2$, and 5.55 glucose (pH 7.4), bubbled with $O_2$ at 37 °C for 5 min. After stabilization, the hearts were perfused with $Ca^{2+}$-free Tyrode's solution for 5 min, then with $Ca^{2+}$-free isolation solution containing 1 mg/ml collagenase II (Sigma, St. Louis, MO) for 30 min. Isolated myocytes were kept in modified Kraft-Brühe solution containing (mM) 30 KCl, 10 $KH_2PO_4$, 0.5 $MgCl_2$, 70 glutamic acid, 10 glucose, 10 HEPES, 15 taurine and 0.5 EGTA (pH 7.4 with KOH). The percentage of viable cells in control and diabetic groups were >70%. Subsequently, cells were kept at 4 °C until used for following experiments.