Association of Ischemic Contracture, Hypercontracture and Post-Ischemic Recovery in Diabetic Rat Heart

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Authors' contributions

This work was carried out in collaboration between both authors. Author HOA managed the experimental process, did the statistical analysis and wrote the first draft of the manuscript. Author ATO managed the experimental process and contributed to critical revision of the manuscript. Final manuscript was done by both authors.

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ABSTRACT

Objective: Previous studies suggest a relation among ischemic contracture and/or hypercontracture profiles and the functional responses in diseased hearts isolated from hypothyroid and post-infarcted animals. We therefore aimed to find out whether there is a similar relation in the diabetic hearts.

Methods: Experiments were performed on Sprague-Dawley rats divided into control and diabetic groups. Diabetes was induced by streptozotocin (45 mg/kg) in citrate buffer (0.1 M, pH:4.5) into the tail vein of the rat. Isolated hearts from control and diabetic rats were subjected to 40-min ischemia followed by 40-min of reperfusion. The left ventricular end-diastolic pressure and left ventricular developed pressure were measured during the experimental protocol. Furthermore, the increase in minimal value of left ventricular pressure during ischemia and the peak value of left ventricular end-

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diastolic pressure measured during the first 5 min of reperfusion for each heart were evaluated as a measure of ischemic contracture and hypercontracture, respectively.

Results: Ischemic contracture did not develop and hypercontracture was markedly suppressed in the diabetic hearts compared with control hearts. In addition, post-ischemic recovery was negatively correlated with hypercontracture in both groups.

Conclusion: Both the non-existence of ischemic contracture and the suppressed hypercontracture results in a better post-ischemic recovery of diabetic heart. Furthermore, the relationship between hypercontracture and post-ischemic recovery suggests that the utility of hypercontracture as a predictor of the post-ischemic recovery.

Keywords: Diabetes; heart; ischemic contracture; hypercontracture; ischemia-reperfusion injury.

1. INTRODUCTION

Evidence shows that ischemic contracture and/or hypercontracture profiles of the hearts are very closely related to the functional responses of those hearts to ischemia/reperfusion (I/R) injury [1-3]. This relation has also been observed in hearts from hypothyroid and post-infarcted animals, similar to the hearts obtained from healthy animals [4,5]. However, there is no data, to our knowledge, regarding the relation in diabetic hearts.

The increase in left ventricular end diastolic pressure during ischemia is known as an indicator of the ischemic contracture [1]. A decline in cytosolic adenosine 5’-triphosphatase (ATP) and accumulation of intracellular calcium (Ca^{2+}) appears to be important factors causing the contracture and irreversible cell damage [6-10]. During ischemia, ATP is provided by anaerobic glycolysis from either glucose or glycogen [11]. Anaerobic glycolysis is beneficial due to glycolytic ATP production, but it has a detrimental effect as a result of accumulation of the deleterious metabolic end products such as protons (H^+) in the ischemic myocardium [12]. The accumulation of H^+ during ischemia may exacerbate myocardial injury by exchanging with sodium (Na^+) through Na^+/H^+ exchanger, especially at the onset of reperfusion [13,14]. This would result in an excess increase in cytosolic Na^+ concentration. Subsequently, Na^+/Ca^{2+} exchange is activated to work in reverse-mode, leading to Ca^{2+} overload and results in myocardial damage [15-18].

Hypercontracture develops within minutes of the onset of reperfusion and is suggested to be associated with uncontrolled Ca^{2+} influx [1,19,20]. In terms of ventricular function, the development of hypercontracture markedly increases end-diastolic pressure and ventricular wall stiffness [19].

The hearts from diabetic animals are known to have some alterations in calcium handling such as reduction of Ca^{2+} influx because of depressed activities of Na^+/H^+ and Na^+/Ca^{2+} exchangers [12,21-24]. Therefore, it may be expected that diabetic hearts have different contracture profiles than controls. In the present study, we aimed to investigate the relation of ischemic contracture and hypercontracture with the post-ischemic recovery of function in the diabetic hearts.

2. MATERIALS and METHODS

2.1 Animals

After the approval of the Institutional Animal Care and Use Committee of Ankara University, 20 Sprague Dawley male rats (300-350 g) were housed under standard conditions of temperature 23 ± 2°C with regular 12 h light/12 h dark cycle and allowed free access to standard laboratory food and water.

2.2 Induction of Experimental Diabetes

Diabetes was induced by single injection of streptozotocin (45 mg/kg, Sigma) (STZ) in citrate buffer (0.1 M, pH:4.5) into the tail vein of rat. Age-matched control rats only received an injection of citrate buffer. Tail vein blood glucose levels were measured using glucometer (Accu-ChekGo, Roche) with test strips (Accu-ChekGo, Roche) after 72 hours of the injection. Rats with blood glucose levels greater than 300 mg/dL were declared as diabetic. At the end of 8 weeks, we also measured body weights, food and water consumptions of both groups.

2.3 Experimental Design

Rats were divided into two main groups: (1) control (n=10) and (2) diabetic (n=10) groups. 8 weeks after the induction of diabetes, hearts
were quickly isolated from control and diabetic rats under anesthesia and rapidly mounted on the Langendorff apparatus. Each heart was perfused retrogradely with Krebs-Henseleit (K-H) buffer (37°C, pH = 7.4). The perfusion buffer was gassed continuously with a 95% O₂ and 5% CO₂ mixture. After excision of atriaus, the hearts were stimulated at 300 beats/min with using Grass S44 stimulator (Grass Instrument. Inc., Quincy, MA, USA). At the beginning of each experiment, a water-filled latex balloon connected to a pressure transducer was inserted into the left ventricle and the left ventricular end-diastolic pressure (LVEDP) was adjusted at 5–10 mmHg. The hearts were firstly perfused at constant pressure (60–65 mmHg) and coronary flow rates were detected with a flowmeter (Transonic Systems Inc., Quincy, MA, USA). Then, the hearts were perfused with their own detected flow rates through a pump (Masterflex Model:77200-12, Cole-Parmer, USA). The left ventricular developed pressure (LVDP), an index of left ventricular systolic pressure, was calculated by subtracting LVEDP from the left ventricular peak pressure. Post-ischemic recovery was obtained by the ratio (expressed as a percentage) of LVDP at the end of reperfusion divided by LVDP immediately before the induction of ischemia. For each heart the increase in minimal value of left ventricular pressure during ischemia was recorded and evaluated as a measure of ischemic contracture [1]. In addition, the peak value of LVEDP measured during the first 5 min of reperfusion was evaluated as hypercontracture, indicating indirect index of intracellular Ca²⁺ handling during reperfusion [1,20].

2.4 Statistical Analysis

The Graph Pad 5 Software was used to conduct the statistical analysis. Data were presented as mean ± SEM. According to the test results of normality test (Kolmogorov-Smirnov), unpaired t test was used to compare two groups. Furthermore, the Pearson's correlation coefficient (r) was used to analyze the relationship between the hypercontracture and post-ischemic recovery. A P value < 0.05 was considered significant.

3. RESULTS

At the end of the 8 weeks, general characteristics of control and diabetic groups are shown in Table 1. The blood glucose levels were significantly higher and body weights were lower in the diabetic group compared with controls (P<0.05). In the diabetic group, a marked polyphagia and polydipsia also occurred (Table 1).

Fig. 1 shows original tracings of alterations in LVP of control and diabetic heart subjected to I/R injury. Similar to the original tracings, in hearts from diabetic rats, any ischemic contracture did not occur whereas there was a marked contracture in hearts from control rats during ischemia (Fig. 2).
Table 1. The blood glucose level, body weight, food and water consumption of control (n=10) and diabetic (n=10) groups at the end of the 8 week

| Group     | Body weight (g) | Blood glucose level (mg/dl) | Food consumption (g/day) | Water consumption (ml/day) |
|-----------|-----------------|----------------------------|--------------------------|---------------------------|
| Control   | 423± 8          | 97 ± 4                     | 20 ± 1                   | 50 ± 3                    |
| Diabetic  | 235 ± 15*       | 348 ± 17*                  | 32 ± 1*                  | 161 ± 15*                 |

*P < 0.05 vs. Control group

As shown in the Fig. 3, hyperconracture in diabetic hearts was significantly lower than that in control hearts (P < 0.05). At the end of reperfusion, LVEDP for control and diabetic hearts are shown in Fig. 3. In the diabetic group, LVEDP was significantly decreased compared with controls (P< 0.05).

Fig. 2. Ischemic contracture profiles of control (n=10) and diabetic hearts (n=10) subjected to 40 min of ischemia

Fig. 3. Hypercontracture (black triangle) and left ventricular end diastolic pressure at the end of reperfusion (white triangle) of control (n=10) and diabetic groups (n=10).

*P < 0.05 vs. Control group
The correlation between the post-ischemic recovery and hypercontracture is shown in the Fig. 4. The post-ischemic recovery showed a negative correlation with hypercontracture both in control (Fig. 4A) and diabetic (Fig. 4B) hearts ($P < 0.05$). Correlation coefficients ($r$) were $r = -0.76$ and $r = -0.80$ in control and diabetic hearts, respectively.

4. DISCUSSION

In the present study, we demonstrated for the first time that hearts from 8-week diabetic rats did not undergo ischemic contracture. In addition, hypercontracture was markedly suppressed in these diabetic hearts. We have also shown that the greater post-ischemic recovery is associated with the lower hypercontracture.

Cardiac changes in critical processes leading to abnormalities in $\text{Ca}^{2+}$ homeostasis is a common phenomenon that occurs under some stress conditions [15]. During I/R, intracellular $\text{Ca}^{2+}$ accumulation in cardiomyocytes is an important factor causing the contracture, thereby impairing cardiac contractility [1,6-10,19,20]. Therefore, our findings showing that there was no contracture during ischemia and suppressed hypercontracture during early phase of reperfusion in the diabetic heart might be associated with diabetes-induced alterations in intracellular $\text{Ca}^{2+}$ handling. Indeed, there is evidence that the rate of sarcolemmal $\text{Ca}^{2+}$ influx into the cells during reperfusion in diabetic heart is lower than that in control heart [24]. In addition, $\text{Na}^+/\text{H}^+$ and $\text{Na}^+/\text{Ca}^{2+}$ exchanger activities have been reported to be depressed in the diabetic rat hearts [12,21-24].

In agreement with our results, diseased myocardiums are shown to have suppressed ischemic contracture and/or hypercontracture profiles with better post-ischemic recovery of function. Pantos et al. [4] showed that the post-infarcted heart was resistant to I/R injury in consistent with the lower ischemic contracture. Hypothyroid hearts were also found to display greater post-ischemic recovery of function accompanied by a suppressed ischemic contracture [5]. Similarly, dronedarone (thyroid hormone receptor $\alpha_1$ antagonist) treatment in rats resulted in a suppressed ischemic contracture and a decreased post-ischemic LVEDP [3]. Zhang et al. [25] reported that hypothyroid state occuring in the STZ-diabetic rats was responsible for the protection against I/R arrhythmias in the diabetic hearts since this protection was found to be abolished by administration of triiodothyronine. Like diabetic state, it has been shown that mRNA expressions of $\text{Na}^+/\text{H}^+$ and $\text{Na}^+/\text{Ca}^{2+}$ exchangers are decreased in the hearts from hypothyroid rats [26].

5. CONCLUSION

In conclusion, the non-existence of ischemic contracture and suppressed hypercontracture improve the post-ischemic contractile recovery in the diabetic heart. In addition, the relationship between hypercontracture and post-ischemic recovery in both groups suggests that the utility of hypercontracture as a predictor of the post-ischemic recovery of the heart subjected to ischemia/reperfusion injury.
DISCLOSURE

Part of this data was presented as a poster in the following conference.

Conference name: Pharmacology 2014,
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Location: London, UK.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the Institutional Animal Care and Use Committee of Ankara University.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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