

## Mechanical effects of ranolazine on normal and diabetic-isolated rat heart

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### Abstract

**Background and purpose:** Diabetic cardiomyopathy is a complication of diabetes defined as cardiac dysfunction without the involvement of pericardial vessels, hypertension, or cardiac valve disorders. Ranolazine, an antianginal drug, acts through blocking of cardiac late sodium channels and/or inhibiting beta-oxidation of fatty acids. With regard to its mechanism of action, the present work has been carried out to investigate the potential useful effects of ranolazine on the systolic and diastolic dysfunctions in an experimental rat model of diabetic cardiomyopathy. Lidocaine, as a sodium channel blocker, was used to have a clearer image of the involved mechanisms.

**Experimental approach:** Diabetes was induced by streptozocin. After 8 weeks, the effects of cumulative concentrations of ranolazine and lidocaine were evaluated on diabetic and normal hearts by the Langendorff method. Finally, the hearts were isolated from the Langendorff system and adenosine three phosphates (ATP) and adenosine diphosphate (ADP) concentrations were measured to assay the metabolic effect of ranolazine.

**Findings/Results:** Ranolazine significantly decreased the velocity of systolic contraction (+dP/dt) and the velocity of diastolic relaxation (-dP/dt) and developed pressure in normal and diabetic rat hearts. However, this negative effect was greater in normal hearts compared to diabetics. Ranolazine (100  $\mu$ M) decreased the ATP level only in normal hearts and the ATP/ADP ratio decreased significantly ( $P < 0.05$ ) in both groups. This reduction was more prominent in normal hearts.

**Conclusion and implications:** It is concluded that in the isolated rat heart preparation, ranolazine has no benefit on diabetic cardiomyopathy and may even worsen it. It seems that these effects are related to the metabolic effects of ranolazine.

**Keywords:** Diabetes; Langendorff isolated heart system; Lidocaine; Ranolazine.

### INTRODUCTION

Diabetes mellitus is the most common metabolic syndrome and its prevalence is increasing all around the world (1). It has been observed that cardiomyopathy is more prevalent in diabetes (2). In fact, it is proved that diabetes can directly affect the cardiac myocytes and this is called diabetic cardiomyopathy and is defined as cardiac dysfunction without the involvement of pericardial vessels, hypertension, or cardiac valve disorders (3). It is manifested as diastolic dysfunction in earlier stages and systolic

dysfunction in later phases. Diastolic dysfunction (decrease in ventricular relaxation rate and increase in diastolic muscular stiffness), and systolic dysfunction (decrease in ventricular contractility rate and peak end systolic pressure) are usually related to intracellular calcium balance disturbance (2,3).

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Ranolazine, an antianginal drug, acts through blocking of cardiac late sodium channels and/or inhibiting of 3-ketoacyl coenzyme A thiolase, an important enzyme in beta-oxidation of fatty acids (4). It is proposed that the inhibition of fatty acids beta-oxidation leads to lower oxygen need of cardiac myocytes to produce ATP and it helps angina to be relieved. Also, inhibition of the sodium entry through the late sodium channels decreases the intracellular sodium and this increases sodium/calcium exchanger activity and accelerates the exit of the intracellular calcium (4). Finally, it facilitates cardiac relaxation, decreases intra-myocardial tension, and improves cardiac blood circulation and angina treatment. According to the mentioned mechanisms, we expected that diastolic dysfunction in diabetic cardiomyopathy improves by ranolazine. On the other hand, this mechanism of ranolazine by the decrease of intracellular calcium stores (an opposite effect of digitalis products) seems to be not effective and even may worsen systolic dysfunction in diabetic cardiomyopathy. There are limited studies on chronic heart failure and the ranolazine effect. In contrast to our expectation, in a study conducted by Sabbah *et al.* one of the few studies in this issue, ranolazine improved the systolic function of dogs with chronic heart failure (5). Also, ranolazine has shown some beneficial effects on contractile dysfunction mediated by increasing myocardial adenosine levels (6). It is also postulated that fatty acid metabolism modulators like ranolazine and trimetazidine might be effective in diabetic cardiomyopathy (7).

In the present study, the effect of ranolazine on diabetic cardiomyopathy and cardiac contractility parameters were evaluated using isolated diabetic rat hearts to investigate the effect and mechanism of ranolazine action on the systolic and diastolic dysfunctions. Also, the effect of ranolazine on the ATP content and ATP/ADP ratio in diabetic rat hearts was measured to assay the metabolic effect of ranolazine. In order to investigate the mechanism of the observed effects of ranolazine, lidocaine as a late sodium channel inhibitor was used. It must be mentioned that lidocaine inhibits the late sodium channel more

than fast (peak) sodium channels (8). However, its selectivity for late types is less than ranolazine. Unlike ranolazine, it does not have any direct specific metabolic effects.

## MATERIALS AND METHODS

### Chemicals

Chemicals were purchased from Sigma (St. Louis, USA) and MERCK (Germany). Ranolazine hydrochloride was purchased from LKT Co. (USA), lidocaine was purchased from Sigma (St. Louis, USA) and both of them were dissolved in distilled water before usage.

### Experimental protocol

Forty-two adult male Sprague-Dawley rats weighing about 200-250 g were obtained from the Animal Care Center of Shiraz University of Medical Sciences. The animals were kept in the laboratory conditions of  $24 \pm 2$  °C with a 12/12-h light/dark alternating cycle with free access to water and a standard diet. The rats were randomly divided into 2 groups of the control and diabetic groups, each containing 21 rats. All applicable institutional guidelines for the care and use of animals were followed. The animal procedures were approved by the Bioethics Committee of Shiraz University of Medical Sciences (Ethics No. IR.SUMS.REC.1389.S5405).

### Induction of diabetes

Diabetes was induced in overnight-fasting rats of the diabetic group by a single intraperitoneal (i.p.) injection of streptozocin (60 mg/kg) dissolved in the citrate buffer (0.1 M, pH 4.5) as the vehicle, whereas the rats in the control group received only the vehicle (1). Fasting blood glucose levels were measured 7 days after streptozocin injection by a home blood glucose test meter (Accu-Chek, Roche Diagnostic, Germany). Rats with blood glucose > 350 mg/dL were considered diabetics. All the rats were kept for 8 weeks in the standard condition. On the 56<sup>th</sup> day, each group was divided into 3 subgroups (with/without treatment) of 7 rats for testing the effects of ranolazine and lidocaine in a mechanical heart assay, as stated below.

### **Isolated rat heart study**

After 8 weeks, the rats were i.p. injected with 1,000 IU/kg heparin, 20 min before being sacrificed. The rats were anesthetized by pentobarbital (70 mg/kg); then, the thorax was opened and the heart was rapidly excised. Aorta was cannulated by a 21-gauge needle and the heart was perfused according to the Langendorff technique (9) in a Langendorff apparatus (ML176-V; ADInstruments, New South Wales, Australia) at constant flow (10 mL/min) with Tyrode's solution with the following composition: NaCl 118, KCl 4.7, CaCl<sub>2</sub> 2.5, MgSO<sub>4</sub> 1.2, NaHCO<sub>3</sub> 25, KH<sub>2</sub>PO<sub>4</sub> 1.2, and glucose 11 mmol/L, equilibrated with 95% O<sub>2</sub> and 5% CO<sub>2</sub> (pH = 7.4). The surface temperature of the heart was 37 °C. The hearts were paced 300 beats/min (voltage 20% more than threshold, 1 ms pulse width). End diastolic pressure was adjusted to 5-10 mmHg by the inflation of an inserted balloon inside the left ventricle. The heart was allowed to be stable for 30 min. There were six groups of rat hearts:

1. Normal hearts were used as a control group to show any spontaneous changes in the measured parameters during the study (40 min).

2. Normal hearts treated with cumulative concentrations of ranolazine (3, 10, 30, and 100 mM) with 10 min intervals between the doses.

3. Normal hearts treated with cumulative concentrations of lidocaine (12.5, 25, 50, and 100 mM) with 10 min intervals between the doses.

4. Diabetic hearts were used as a control group to show any spontaneous changes in the measured parameters during the study (40 min).

5. Diabetic hearts treated with ranolazine (3, 10, 30, and 100 mM) with 10 min intervals between the doses.

6. Diabetic hearts treated with cumulative concentrations of lidocaine (12.5, 25, 50, and 100 mM) with 10 min intervals between the doses.

Before the addition of each dose, the velocity of systolic contraction (+dP/dt), the velocity of diastolic relaxation (-dP/dt), and developed pressure (DP) were recorded, using a PowerLab recorder and LabChart 5.0

software (ADInstruments, New South Wales, Australia).

The parameters were also recorded and calculated in the control hearts in the times corresponding to the time of the addition of drugs to the treated hearts. Cumulative concentrations of ranolazine and lidocaine were chosen based on the previously published studies and relevant to the IC<sub>50</sub> of each treatment (8, 10, 11).

### **ATP and ADP assay**

At the end of the isolated heart study and 10 min after the addition of the last concentrations of ranolazine, lidocaine, or vehicle, the hearts were detached from the apparatus and immediately the isolated hearts were kept in liquid nitrogen. ATP and ADP levels were quantified using ATP-dependent luciferin-luciferase bioluminescence assay by the luminometer (Berthold LB 9501, Germany) in terms of nmol/mg protein (Bioluminescence Somatic Cell Assay System; Sigma, USA).

### **Data analysis**

All values are given as mean ± SEM of experiments. Appropriate statistical analyses including 2-way ANOVA followed by Tukey's post hoc test and Mann-Whitney U were used to compare the differences between with or without treatments in each group using SPSS software Ver 16. *P* < 0.05 was considered statistically significant.

## **RESULTS**

### **Induction of diabetes**

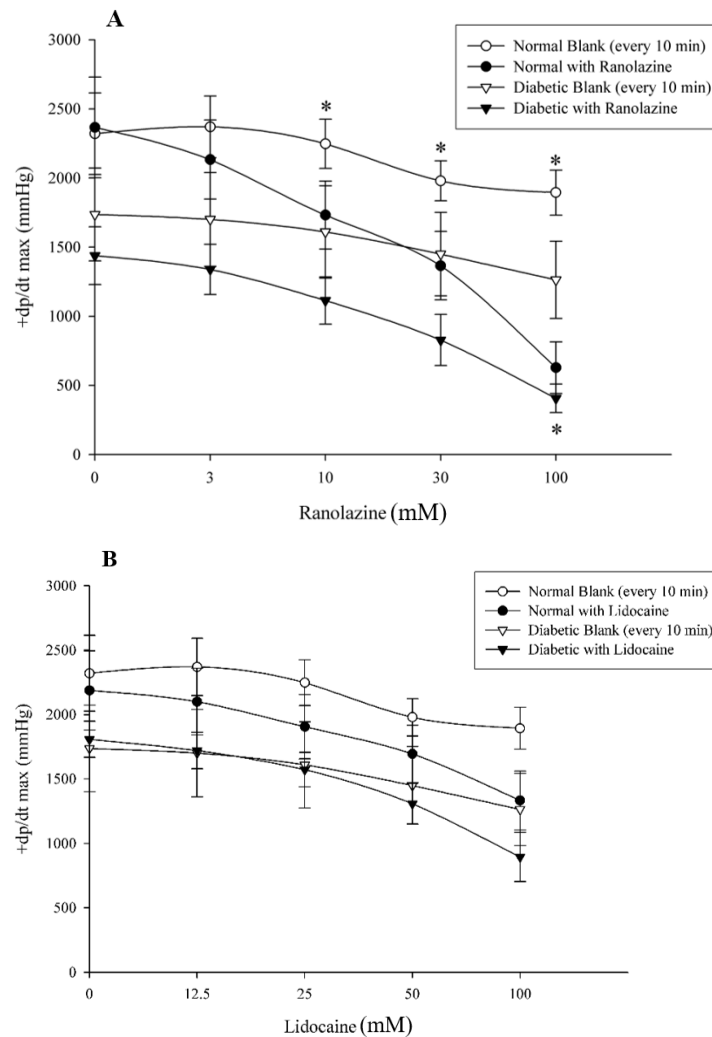
Blood glucose of diabetic rats was significantly higher than normal animals. Diabetic animals also showed significant weight loss after 8 weeks of the induction of diabetes (Table 1).

### **Isolated rat heart study**

+dP/dt, -dP/dt, and DP decreased significantly in diabetic hearts, indicating the successful induction of diabetic cardiomyopathy (Table 1).

**Table 1.** Effect of diabetes on blood glucose, body weight, mechanical, and metabolic parameters of the isolated heart. Data showed mean ± SEM.

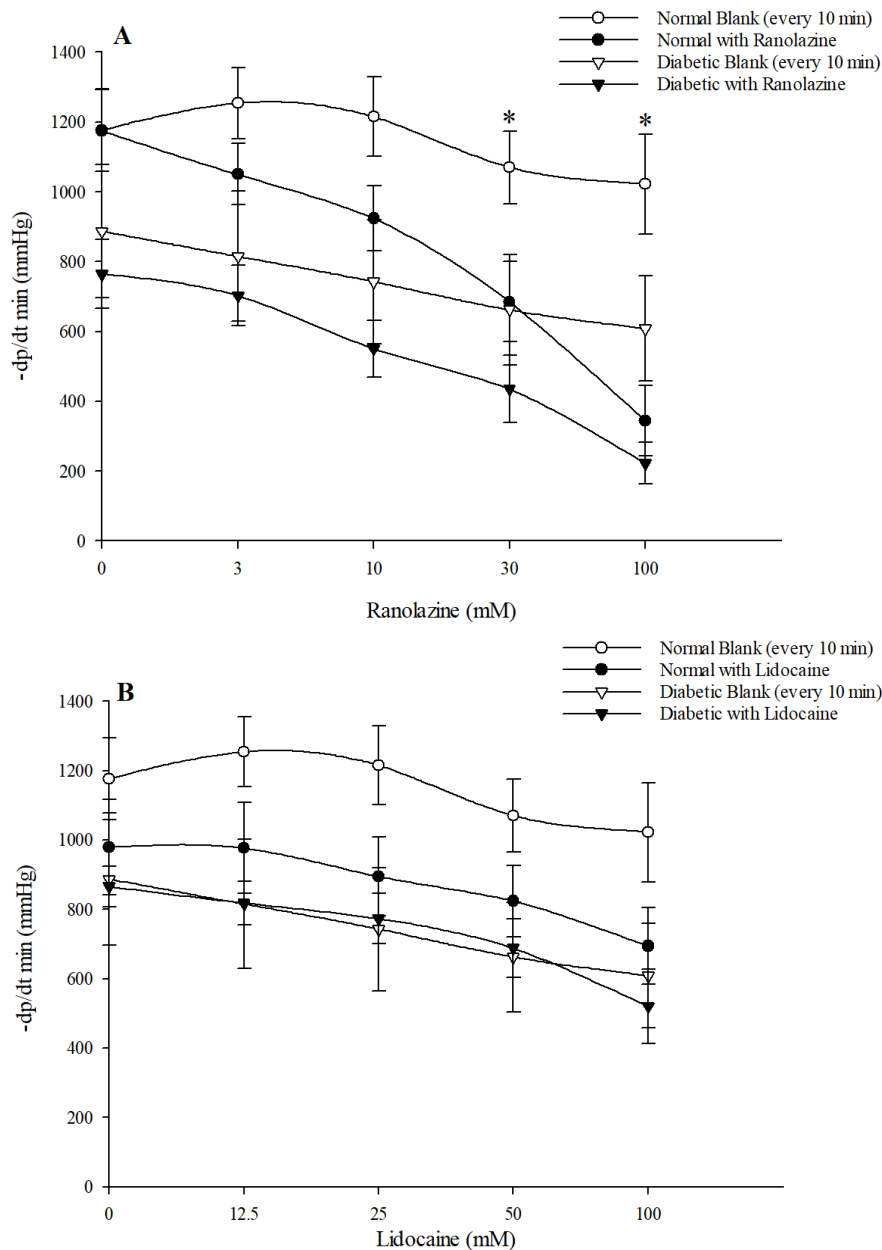
Groups	Blood glucose (mg/dL)	Weight (g)	Weight heart/body	-dp/dt Ventricle (mmHg/s)	+dp/dt Ventricle (mmHg/s)	Developed pressure (mmHg)	ADP (μM)	ATP (μM)	Ratio ATP/ADP
Normal	120.4 ± 5.4	304.0 ± 9.8	(3.6 ± 0.2) × 10 <sup>-3</sup>	1160 ± 76	2409 ± 193	3.5 ± 55.9	5.11 ± 0.6	23.54 ± 1.0	5.05 ± 0.7
Diabetic	517.8 ± 9.5	220.0 ± 10.9	(5.4 ± 0.5) × 10 <sup>-3</sup>	886 ± 66	1770 ± 133	3.4 ± 48.2	13.37 ± 0.9	14.04 ± 0.3	1.08 ± 0.1
Significance	<i>P</i> < 0.001	<i>P</i> < 0.01	<i>P</i> < 0.05	<i>P</i> < 0.05	<i>P</i> < 0.05	NS <i>P</i> = 0.1	<i>P</i> < 0.001	<i>P</i> < 0.001	<i>P</i> < 0.001



**Fig. 1.** Effect of cumulative concentrations of (A) ranolazine and (B) lidocaine on +dP/dt in normal and diabetic hearts. Applied concentrations were added every 10 min. Each point represents the mean ± SEM, n = 7. \**P* < 0.05 Indicates a significant difference between treatment and without treatment at each point in each group.

Ranolazine significantly decreased +dP/dt in normal hearts (*P* = 0.007) compared with non-treated normal hearts and in diabetic hearts (*P* = 0.006) compared with non-treated diabetic hearts. Also, this negative effect was greater in normal hearts compared to diabetics (*P* = 0.001). Lidocaine had no significant effect on +dP/dt in both normal and diabetic hearts (Fig. 1).

Ranolazine significantly decreased -dP/dt in normal hearts (*P* = 0.001) and in diabetic hearts (*P* = 0.045). Also, this negative effect was greater in normal hearts compared to diabetics (*P* = 0.001). Lidocaine significantly decreased (*P* = 0.005) -dP/dt in normal hearts but had no significant effect on diabetic hearts (Fig. 2).



**Fig. 2.** Effect of cumulative concentrations of (A) ranolazine and (B) lidocaine on  $-dp/dt$  in normal and diabetic hearts. Applied concentrations were added every 10 min. Each point represents the mean  $\pm$  SEM,  $n = 7$ . \* $P < 0.05$  shows a significant difference between treatment and without treatment at each point in each group.

Ranolazine significantly decreased DP in normal hearts ( $P = 0.02$ ) and in diabetic hearts ( $P = 0.008$ ). It seemed that its negative effect was greater in normal hearts compared to diabetics but it did not reach a statistical significance ( $P = 0.059$ ). Lidocaine had no significant effect on DP in both normal and diabetic hearts (Fig. 3).

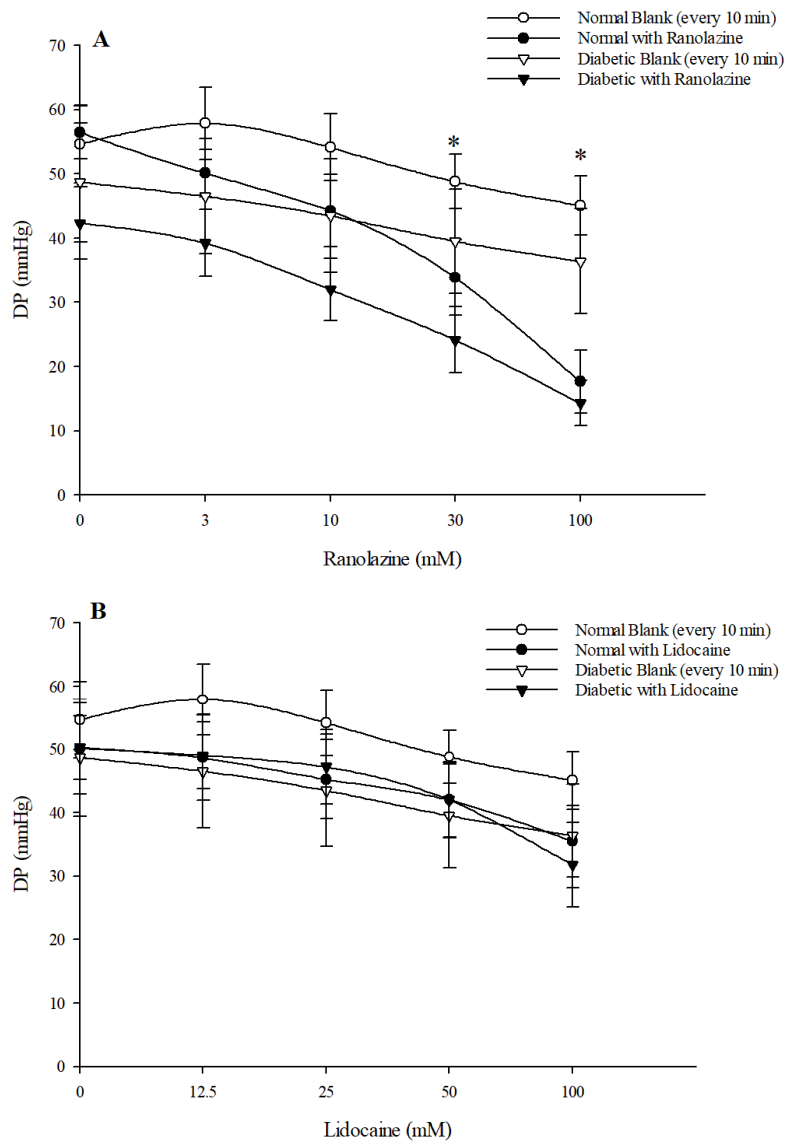
**ATP and ADP contents of the heart**

A non-significant decrease in the ATP content was observed in the diabetic hearts. ATP/ADP was much lower (about 20%) in the

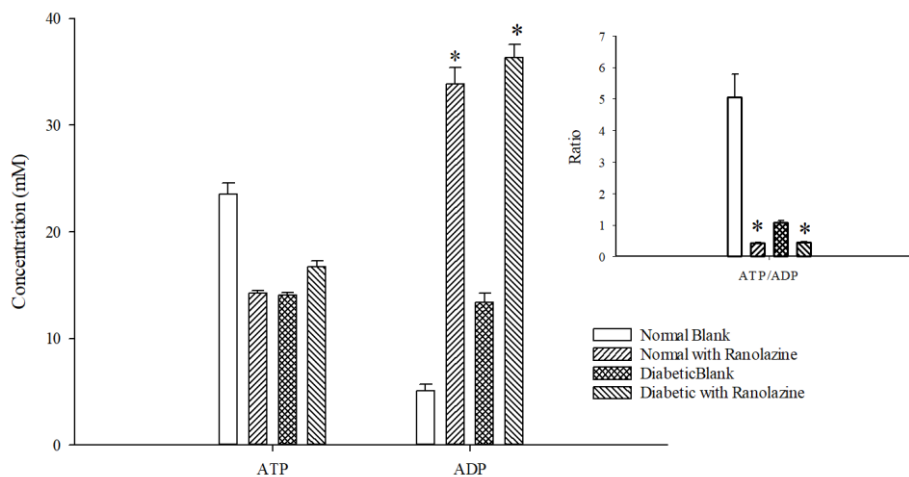
diabetic hearts compared to the normal ones ( $P < 0.01$ ).

ATP content of the normal hearts treated with cumulative concentrations of ranolazine was lower compared to the normal control hearts. In the diabetic hearts, ranolazine did not show any effect on the ATP content compared to the diabetic control hearts ( $P > 0.05$ ; MannWithney U test).

In both normal and diabetic hearts, ranolazine reduced ATP/ADP significantly compared to the normal and diabetic controls, respectively (Fig. 4).



**Fig. 3.** Effect of cumulative concentrations of (A) ranolazine and (B) lidocaine on the left ventricle DP in normal and diabetic hearts. Applied concentrations were added every 10 min. Each point represents the mean  $\pm$  SEM, n = 7. \* $P$  < 0.05 shows a significant difference between treatment and without treatment at each point in each group. DP, Developed pressure



**Fig. 4.** Effect of ranolazine (100  $\mu$ M) on ATP, ADP, and ATP/ADP ratio in homogenized normal and diabetic hearts. Each point represents mean  $\pm$  SEM, n = 7. \* $P$  < 0.05 indicates a significant difference between treatment and without treatment in each group.

## DISCUSSION

In the present study, diabetes decreased the rate of contraction (+dP/dt) and relaxation (-dP/dt) of isolated hearts, indicating that diabetic cardiomyopathy had been produced successfully (Table 1). It also significantly decreased the developed pressure of the isolated hearts. Also, a non-significant decrease in the cytosolic ATP level and a significant decrease in ATP/ADP ratio of diabetic cardiomyocytes were observed (Fig. 4).

The obtained metabolic results are similar to those reported in another study in which 24% less ATP and 40% more ADP production and a significant decrease in ATP/ADP ratio in diabetic rat hearts have been reported (12). It seems that mechanical changes in diabetic cardiomyopathy, at least in part, have a metabolic basis of the inability of cardiomyocytes to produce enough energy.

According to our previous study, there was no significant difference among basal activities of the enzymes involved in the fatty acid beta-oxidation pathway in normal and diabetic cardiomyocytes (11). In one study, myocardial ATP levels decreased and subsequent ATP-producing capacity in diabetic hearts with cardiomyopathy was low as compared with those in normal or diabetic rat hearts without cardiomyopathy, especially in subsarcolemmal mitochondria (13).

Mitochondria occupy 35-40% of the volume of the mammalian cardiomyocyte and 95% of the heart's ATP is supplied by mitochondrial oxidative phosphorylation. However, the exact high energy phosphate fluxes and decrease in cardiac pump function in diabetic cardiomyopathy is not clear. Due to the lower capacity and efficiency in respiration, diabetic cardiac mitochondria produce less ATP (14). Mitochondrial dysfunction and lower oxidative phosphorylation decreased the myocardium energy production capacity and caused contractility failure. In addition, more fatty acid oxidation and decreased ATP synthesis deregulate  $\text{Ca}^{2+}$  homeostasis, decrease ionic  $\text{Na}^+/\text{Ca}^{2+}$  exchanger or sarcoplasmic Ca-ATPase activity and impair the function of sarcoplasmic reticulum ability in calcium reuptake, and slow the heart relaxation (3,14).

In diabetic rat hearts, down-regulated expression of sarcoplasmic reticulum  $\text{Ca}^{2+}$ -ATPase 2a (SERCA2a) was found, and also steady-state ATP synthesis capacity was almost one-third lower than the normal hearts (14).

Higher demands of the hearts led to a further decrease in ATP/ADP and increase in systolic dysfunction, but the concentration of ATP was not decreased as high as ATP/ADP ratio in the hearts, and an increase in the ADP level was shown, similar to our results. Altered substrate preference from fatty acids to glucose might contribute to the increased ADP concentration in diabetic hearts. On the other hand, elevated ADP level or other factors such as oxidative modifications inhibited ATPase and decreased ATP utilization.

We hypothesized that if ranolazine inhibited fatty acids beta-oxidation, the need for oxygen would reduce and diastolic and systolic dysfunction of diabetic cardiomyopathy would improve. Also, if ranolazine inhibited late sodium channels, it would have a positive effect at least on diastolic dysfunction in diabetic cardiomyopathy. However, with the later proposed mechanism, it might not have a positive effect on systolic dysfunction or even made it worse.

Ranolazine significantly decreased +dP/dt and -dP/dt and developed pressure in normal and diabetic hearts (Figs. 1-3). However, these effects were more prominent in the normal heart according to the significant results of different concentrations. We also studied the effect of lidocaine as a mixed blocker of both fast and late sodium channels. Lidocaine did not show a significant effect on +dP/dt, -dP/dt and DP in both normal and diabetic hearts. (Figs. 1-3).

The above observations showed that ranolazine had no direct beneficial effect on diabetic cardiomyopathy and may even worsen it. Moreover, it had a negative effect on the contractility of the isolated normal hearts. Also, it seems that the blocking of late sodium channels may have no major role in its effect on the contractility of the normal hearts because those effects, both qualitatively and quantitatively, were not observed with lidocaine. Thus, we suggest that the effects of ranolazine may have a metabolic basis. In fact,

ranolazine unexpectedly decreased the ATP production and ATP/ADP ratio significantly and on a great scale. Although it had the same effect on diabetic cardiac tissue, its effects on the diabetic hearts were much smaller than those observed in the normal hearts (similar to its action on the mechanical activity of the heart). It must be mentioned that in our study the ATP concentration and ATP/ADP ratio were measured in the cardiac muscle that had been treated with cumulative concentrations of ranolazine. In other words, the data obtained showed the effect of ranolazine with its highest concentration, i.e. 100  $\mu$ M, and we have no data on the effects of lower concentrations on the ATP level and ATP/ADP ratio. While many researchers believe that by the inhibition of the metabolism of fatty acids, ranolazine shifts the cardiac energy production to the carbohydrate metabolism and providing more ATP at the same level of oxygen, the reverse was observed in our experiments. We have no explanation for the observed results, but it can be proposed that the setting of ischemia (which is the subject of most animal studies) is different from a specific kind of cardiomyopathies (i.e. diabetic cardiomyopathy), and maybe this is the basis of the differences among obtained results.

## CONCLUSION

In conclusion, in the isolated rat heart preparation, ranolazine has no benefit on diabetic cardiomyopathy and may even worsen it. It also weakens all mechanical parameters of the isolated normal hearts. It is suggested that these effects are mainly related to metabolic effects of ranolazine on the ATP/ADP ratio and it seems that late sodium current inhibition has no a great and important role in this regard.

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## Conflict of interest statement

The authors declared no conflict of interest in this study.

## Authors' contribution

All authors contributed equally to this work.

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