Experimental iron deficiency in rats: mechanical and electrophysiological alterations in the cardiac muscle

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(Received 15 January/18 April 1996; accepted 29 April 1996)

1. Our aim was to investigate the effect of experimental iron deficiency on cardiac functional properties. We recorded ventricular isometric twitch, action potentials and the L-type Ca$^{2+}$ current in isolated ventricular myocytes from iron-deficient rats and control rats.

2. Twitch tension and maximal rates of tension activation and relaxation were reduced in iron-deficient compared with control rats, whereas twitch duration was prolonged. Isoproterenol (10$^{-4}$ mol/l) augmented tension in iron-deficient rats ($P<0.05$), but only moderately affected control rats. In contrast, maximal rates of tension activation and relaxation were increased equally by isoproterenol in the two groups.

3. To determine the mechanism(s) responsible for the reduced mechanical function in iron-deficient rats, action potentials and the L-type Ca$^{2+}$ current (with or without isoproterenol) were recorded in both groups.

4. The L-type Ca$^{2+}$ current was smaller in ventricular myocytes from control rats than in those from iron-deficient rats; at a membrane potential of 0 mV, L-type Ca$^{2+}$ current amplitudes were $-1.44 \pm 0.18$ and $-0.97 \pm 0.07$ nA in myocytes from control and iron-deficient rats respectively ($P<0.05$).

5. Action potential duration was markedly shortened in myocytes from iron-deficient compared with control rats; action potential duration at 50% repolarization was 12.0 ± 1.6 and 7.2 ± 1.4 ms in myocytes from control and iron-deficient rats respectively ($P<0.01$). These iron deficiency-induced electrophysiological alterations most probably contribute to the depressed mechanical function in iron-deficient rats.

6. The L-type Ca$^{2+}$ current was augmented equally by isoproterenol in the two groups, suggesting that the enhanced inotropic responsiveness in iron-deficient rats was not due to an increased response of the L-type Ca$^{2+}$ current.

7. These results may have an important implication for anaemic (iron-deficient) patients; the attenuation of their cardiac mechanical performance may be compensated by an increased reactivity to β-adrenergic stimulation.

INTRODUCTION

Iron deficiency, the most common nutritional deficiency [1], is associated with deleterious effects on a wide range of body functions, such as exercise and work performance, immune function, and catecholamine and thyroid hormone metabolism [1, 2]. Iron deficiency also affects major cardiovascular structural and functional properties [3, 4]. Clinical studies have demonstrated increased cardiac output resulting from decreased blood viscosity and total peripheral resistance [5–7], in addition to increased formation of intercoronary collaterals and preload, as well as reduction in afterload [8]. Kovalenko and Kuzmina [9] demonstrated right ventricular dilatation and increased end-systolic volume in patients with severe iron-deficiency anaemia. In patients with chronic anaemia, increased left ventricular end-diastolic volume has been demonstrated [10]. The enhanced left ventricular function in chronic anaemia has also been attributed to increased catecholamine levels and non-catecholamine inotropic factors in the plasma [11, 12]. For example, papillary muscles exposed to serum obtained from patients with chronic anaemia exhibited increased contractility [12].

Although the effects of iron deficiency on the overall cardiovascular system have been well documented, less is known about iron deficiency-induced changes in the functional properties of the cardiac muscle. Thus, in the study described here, we investigated the effects of experimental iron deficiency on the mechanical and electrophysiological properties of papillary muscles and isolated ventricular myocytes. Specifically, cardiac mechanical function was assessed by recording the isometric twitch from electrically stimulated right ventricular papillary muscle preparations, and membrane potential and currents were recorded from isolated ventricular...
myocytes by means of the 'whole-cell' configuration. In addition, iron deficiency-induced changes in the $\beta$-adrenergic inotropic responsiveness were determined by comparing the effect of isoproterenol in control and iron-deficient rats.

**MATERIALS**

**Animals and experimental model**

The study was performed on two groups of adult male Sprague–Dawley rats: control and iron deficient. Rats aged 21 days were fed a regular diet containing 250 p.p.m. (NH$_4$)$_2$Fe(SO$_4$)$_2$ or an iron-deficient diet with a low iron content (5 p.p.m. iron) [13]. Rats were killed after 7–8 weeks on the diet. At weekly intervals, rats were weighed, and tail blood samples were obtained to determine haemoglobin values spectrophotometrically, using a Sigma kit [14].

**Measurements of mechanical function in papillary muscles**

Rats were anaesthetized with pentobarbitone sodium, 30 mg/kg, injected intraperitoneally. The heart was rapidly removed, and the right ventricle excised and placed in cold Tyrode's solution (4–6°C) gassed with 95% oxygen and 5% carbon dioxide. A thin (<1 mm in diameter) papillary muscle was removed from the right ventricle and mounted horizontally in a Lucite tissue bath superfused at a rate of 11 ml/min with Tyrode's solution gassed with 95% oxygen and 5% carbon dioxide and warmed to 36.0±0.2°C [15]. The Tyrode's solution contained (mmol/l): NaCl, 140; MgCl$_2$, 1; Hepes, 5; MgCl$_2$, 0.5; CaCl$_2$, 1.8; glucose, 10; and KCl, 4; pH 7.4. The KB medium contained (mmol/l): KCl, 70; MgSO$_4$, 5; K$_2$HPO$_4$, 30; MgCl$_2$, 0.5; CaCl$_2$, 0.12; glucose, 20; taurine, 20; succinic acid, 5; pyruvic acid, 5; creatine, 5; disodium ATP, 5; and EGTA, 0.5; pH 7.4. Animal studies were ethically approved by the Technion Animal Care Ethics Committee.

After an incubation period (1–2 h) in the KB solution, myocytes were transferred to the recording bath (0.5 ml), mounted on the stage of an inverted microscope (Nikon, Diaphot-TMD, Tokyo, Japan). The bath was superfused with modified Tyrode's solution (unless otherwise indicated) at a rate of 1–2 ml/min. Experiments were carried out at room temperature (24–25°C). Patch electrodes were prepared from soft glass micropipettes (Jencons Scientific, H15/10, Bedfordshire, U.K.) pulled on a vertical puller (Narashige pp-83, Tokyo, Japan), and had a tip resistance of 2–4 MΩ when filled with the pipette solution containing (mmol/l): potassium aspartate, 120; KCl, 20; MgCl$_2$, 3.5; K$_2$HPO$_4$, 20; disodium ATP, 3; glucose, 10; and EGTA, 1; pH 7.4.

Membrane potentials and currents were measured by means of the whole-cell recording [18] with the Axon 200A patch clamp amplifier (Foster City, CA, U.S.A.). The following action potential characteristics were analysed: action potential amplitude (APA), resting membrane potential ($V_m$) and action potential duration (APD) at 10% and 50% repolarization (APD$_{10}$ and APD$_{50}$ respectively). The PCLAMP software package was used for generating voltage clamp protocols, data acquisition and storage and off-line analysis. The L-type Ca$^{2+}$ current ($I_{Ca,L}$) was measured as the amplitude of the inward

Electrophysiological measurements in isolated ventricular myocytes

Ventricular myocytes were prepared according to procedures described elsewhere [16]. Rats were anaesthetized with sodium pentobarbitone, 30 mg/kg, intraperitoneally. The chest was opened, the heart rapidly removed and the aorta cannulated and perfused with Tyrode's solution in a Langendorff apparatus [17]. After the blood was washed out, the heart was perfused with 50 ml of Ca$^{2+}$-free Tyrode's (CFT) solution. Subsequently, CFT solution containing 0.05–0.06% collagenase (Type II, Worthington Biochemical, Freehold, NJ, U.S.A.) was circulated for 25–35 min. Thereafter the heart was perfused with 50 ml of Kraftrühe (KB) medium [16]. All solutions were oxygenated and warmed to 36.0±0.2°C. Finally, the heart was removed from the cannula, placed in KB medium and the suspension filtered through a nylon mesh and stored in KB medium at room temperature (24–25°C) before the experiment. The modified Tyrode's solution contained (mmol/l): NaCl, 140; MgCl$_2$, 1; Hepes, 5; MgCl$_2$, 0.5; CaCl$_2$, 1.8; glucose, 10; and KCl, 4; pH 7.4. The KB medium contained (mmol/l): KCl, 70; MgSO$_4$, 5; K$_2$HPO$_4$, 30; MgCl$_2$, 0.5; CaCl$_2$, 0.12; glucose, 20; taurine, 20; succinic acid, 5; pyruvic acid, 5; creatine, 5; disodium ATP, 5; and EGTA, 0.5; pH 7.4. Animal studies were ethically approved by the Technion Animal Care Ethics Committee.
Nutritional iron deficiency and the heart

**Tables**

**Table 1. Haemoglobin and thyroid hormone concentrations in control and iron-deficient rats.** Haemoglobin concentrations at weeks 2-4 in the iron-deficient group were statistically different from those during the first week: *P<0.001 (Tukey-Kramer test). **P<0.01, iron-deficient rats versus control rats.

<table>
<thead>
<tr>
<th>Haemoglobin levels (g%)</th>
<th>Free T₃ (pmol/l)</th>
<th>Total T₃ (ng/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 1</td>
<td>Week 2</td>
<td>Week 3</td>
</tr>
<tr>
<td>Control</td>
<td>12.85±0.15</td>
<td>14.0±0.2</td>
</tr>
<tr>
<td>(n=20)</td>
<td>(n=20)</td>
<td>(n=20)</td>
</tr>
<tr>
<td>Iron deficiency</td>
<td>13.5±0.2</td>
<td>5.2±0.2*</td>
</tr>
<tr>
<td>(n=15)</td>
<td></td>
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</table>

**Table 2. Summary of heart/body weight and ventricular myocytes morphometric parameters in control and iron-deficient rats.** For morphometric studies, each group included four rats in which 40 myocytes were measured. *P<0.01.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Iron deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>158±3* (n=14 rats)</td>
<td>151±4 (n=6 rats)</td>
</tr>
<tr>
<td>Heart/body (mg/100g)</td>
<td>563±24* (n=4 rats)</td>
<td>56±3 (n=15 rats)</td>
</tr>
<tr>
<td>Length (μm)</td>
<td>121.1±4</td>
<td>140.6±4.9*</td>
</tr>
<tr>
<td>Width (μm)</td>
<td>18.2±0.8</td>
<td>23.2±1.7*</td>
</tr>
<tr>
<td>Circumference (μm)</td>
<td>281.0±10.2</td>
<td>328.4±9.5*</td>
</tr>
<tr>
<td>Area (μm²)</td>
<td>2149±169</td>
<td>3055±207*</td>
</tr>
</tbody>
</table>

component of the membrane current. $I_{\text{Ca,L}}$ current-voltage ($I-V$) relations were generated by clamping ventricular myocytes from a holding potential of $-40\,\text{mV}$ to series of voltage clamp pulses with a duration of 500 ms and amplitude ranging from $-30\,\text{mV}$ to $+60\,\text{mV}$. The fast inward Na⁺ current ($I_{\text{Na}}$) was eliminated from the membrane current by using a holding potential of $-40\,\text{mV}$, which causes complete inactivation of $I_{\text{Na}}$. Commonly, the delayed outward K⁺ current ($I_{\text{K}}$) is removed by substituting K⁺ for Cs⁺ in the bathing and the pipette solution. As iron-deficient myocytes did not tolerate the Cs⁺-containing solutions, this procedure was not used. Nevertheless, as $I_{\text{K}}$ develops much more slowly than $I_{\text{Ca,L}}$, its amplitude at the beginning of the current response (where $I_{\text{Ca,L}}$ is maximal) is negligible. $I_{\text{Ca,L}}$ was initially measured in Tyrode's solution containing $10^{-3}\,\text{mol/l}$ ascorbic acid (results were unchanged compared with regular Tyrode's solution) and then at $10^{-4}\,\text{mol/l}$ isoproterenol. As the maximal response to isoproterenol occurred within 1–2 min, ‘run-down’ of $I_{\text{Ca,L}}$ was not considered to be of significance in these studies.

**Measurements of ventricular myocyte dimensions**

Length, width, circumference and area were measured from isolated ventricular myocytes, which were photographed via the inverted microscope. All measurements were performed by means of a computerized system (Cue-2 Image Analysis Olympus Corporation, Lake Success, NY, U.S.A.) with Morphometry Software version 1.0 (Galai Corporation, Migdal Haemek, Israel). For each rat, 40 myocytes were analysed.

**Thyroid hormone levels**

Serum total T₃ (TT₃) and free T₄ (FT₄) concentrations in control and iron-deficient rats were measured using commercial kits from Amersham International U.K. Serum was prepared from blood obtained from both groups, and stored at $-20\,\text{°C}$ until thyroid hormone determination.

**Data analysis**

Isometric twitch characteristics from control versus iron-deficient groups were compared using the Student $t$-test for unpaired observations. The curves depicting isoproterenol dose–response curves in both groups were compared by means of the two-way analysis of variance (ANOVA) test. Results are expressed as means±SEM. Membrane current amplitudes in control and iron-deficient myocytes were compared using two-way ANOVA ($P<0.05$).

**RESULTS**

**Evaluation of the experimental model**

The major biochemical index of iron deficiency is marked reduction in haemoglobin concentrations (Table 1). From the second week onwards, haemoglobin concentrations in iron-deficient rats were reduced to below 4g% at 4 weeks ($P<0.001$, compared with control). In addition, thyroid hormone concentrations (TT₃ and FT₄), important markers of iron deficiency, were also significantly ($P<0.01$) reduced in iron-deficient rats (Table 1).

An important morphological consequence of iron deficiency is cardiac hypertrophy [4, 19], demonstrated here as doubling of heart/body weight ratio (Table 2). To evaluate iron deficiency-induced hypertrophy further, we measured length, width, circumference and area of isolated ventricular myocytes from both groups (Table 2). It is seen that all four parameters were markedly increased in iron-deficient rats.

**Effect of iron deficiency on muscle contraction and β-adrenergic responsiveness**

The effects of iron deficiency on ventricular mechanical properties are demonstrated by the rep-
Fig. 1. Representative twitch recordings from papillary muscles of control (upper traces) and iron-deficient (lower traces) rats in the absence (left traces) and the presence (right traces) of 10^{-6}mol/l isoproterenol

Table 3. Summary of isometric twitch characteristics in papillary muscles from control and iron-deficient rats. Tension, maximal twitch tension; +dT/dt, maximal rate of tension activation; −dT/dt, maximal rate of tension relaxation; TTPT, time to peak tension; duration, twitch duration. *P<0.05, **P<0.01.

<table>
<thead>
<tr>
<th></th>
<th>Control (n=8)</th>
<th>Iron deficiency (n=10)</th>
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<tbody>
<tr>
<td>Tension (g/mm²)</td>
<td>1.24±0.08</td>
<td>0.35±0.05**</td>
</tr>
<tr>
<td>+dT/dt (gs^{-1}mm^{-2})</td>
<td>29.8±3.6</td>
<td>13.1±3.4*</td>
</tr>
<tr>
<td>−dT/dt (gs^{-1}mm^{-2})</td>
<td>13.9±0.9</td>
<td>4.6±0.8**</td>
</tr>
<tr>
<td>TTPT (ms)</td>
<td>57.4±4.8</td>
<td>53.0±2.0</td>
</tr>
<tr>
<td>Duration (ms)</td>
<td>147.4±6.8</td>
<td>171.4±3.7*</td>
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As global cardiac function, in health as well as in disease states, is influenced by its ability to respond to β-adrenergic stimulation, we tested the inotropic responsiveness to isoproterenol. As seen in Fig. 1, 10^{-6}mol/l isoproterenol caused a moderate increase in twitch amplitude (13% and 37% in control and iron-deficient preparations respectively) and a more pronounced effect on maximal rates of tension activation and relaxation and twitch duration. The effect of isoproterenol (10^{-9} to 10^{-6} mol/l) on three twitch characteristics is illustrated in Fig. 2. Whereas twitch tension (Fig. 2) was slightly increased in control rats, in iron-deficient rats it was markedly augmented by high concentrations of isoproterenol (P<0.05). Maximal rates of tension activation and relaxation were increased almost equally by isoproterenol in the two groups.

Electrophysiological properties of ventricular myocytes: alterations by iron deficiency

To explore the mechanism(s) responsible for the differences in twitch characteristics and for the altered β-inotropic responsiveness, we recorded action potentials and I_{Ca,L} in the absence and
Nutritional iron deficiency and the heart

Control Iron deficiency

![Graph](Image)

**Fig. 3.** Representative action potentials recorded from ventricular myocytes from control and iron-deficient rats. Cycle length = 2s.

![Graph](Image)

**Fig. 4.** Effect of iron deficiency on the L-type Ca\(^{2+}\) current (\(I_{Ca,L}\)) and responsiveness to isoproterenol in ventricular myocytes from control (squares) and iron-deficient (circles) rats. The current-voltage (I-V) relationship of \(I_{Ca,L}\) was measured as the amplitude of the inward component of the membrane current. \(I_{Ca,L}\) current-voltage (I-V) relations were generated by clamping ventricular myocytes from a holding potential of -40 mV to series of voltage clamp pulses with a duration of 500 ms and amplitude ranging from -30 mV to +60 mV. \(I_{Ca,L}\) was initially measured in Tyrode's solution containing 10\(^{-4}\) mol/l ascorbic acid (results were unchanged compared with regular Tyrode's solution) and then at 10\(^{-5}\) mol/l isoproterenol. Maximal response to isoproterenol occurred within 1-2 min of exposure.

**Table 4.** Action potential characteristics in ventricular myocytes of control and iron-deficient rats. APD\(_{10}\) and APD\(_{50}\) action potential duration at 10% and 50% repolarization. *P < 0.01.

<table>
<thead>
<tr>
<th></th>
<th>Control ((n=10))</th>
<th>Iron-deficient ((n=5))</th>
</tr>
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<tbody>
<tr>
<td>Resting potential (mV)</td>
<td>-77.6 ± 0.4</td>
<td>-75.8 ± 0.7</td>
</tr>
<tr>
<td>Action potential amplitude (mV)</td>
<td>120 ± 4</td>
<td>132 ± <em>3</em></td>
</tr>
<tr>
<td>APD(_{10}) (ms)</td>
<td>2.3 ± 0.4</td>
<td>0.7 ± 0.2*</td>
</tr>
<tr>
<td>APD(_{50}) (ms)</td>
<td>12.0 ± 1.6</td>
<td>7.2 ± 1.4*</td>
</tr>
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</table>

Discussion

Our main objective was to investigate the effects of experimental iron deficiency on the mechanical and electrophysiological properties of the ventricular muscle and its responsiveness to \(\beta\)-adrenergic inotropic stimulation. Specifically, we investigated the alterations induced by iron deficiency in isotonic twitch as well as in action potential and \(I_{Ca,L}\). The main findings were (i) in iron-deficient rats twitch tension and maximal rates of activation and relaxation were decreased, whereas twitch duration was increased; (ii) the positive inotropic response to isoproterenol was augmented in iron-deficient rats; and (iii) basal \(I_{Ca,L}\) amplitude was smaller in iron-deficient than in control rats, whereas the response of both groups to isoproterenol was similar.

Several mechanisms may have contributed to the depressed cardiac mechanical performance in iron-deficient rats. The first mechanism to be discussed is hypothyroidism, reflected by the reduced concentrations of FT\(_4\) and TT\(_3\). Three distinct effects of thyroid hormones may be responsible for the attenuated mechanical function of iron deficient hearts: (1) hypothyroidism causes a shift in myosin ATPase, from the fast isoform V1 to the slow isoform V3 [20], resulting in slower tension development; (2) the reduction in twitch tension and maximal rate of tension relaxation may have resulted from the effect of thyroid hormone on the Ca\(^{2+}\)-pumping capacity of the sarcoplasmic reticulum (hypothyroidism reduces mRNA levels of sarcoplasmic/endoplasmic reticulum calcium ATPase, thereby attenuating tension relaxation rate [21]); (3) thyroid hormones modulate contraction through their effect on \(I_{Ca,L}\). The reduction of \(I_{Ca,L}\) in iron-deficient rats (from \(-1.4 ± 0.18\) to \(-0.97 ± 0.07\) nA) is in agreement with

presence of isoproterenol. Representative action potential traces from both groups are depicted in Fig. 3. The major finding (summarized in Table 4) was a marked shortening of action potential duration in iron-deficient rats, which may cause negative inotropism. This effect could contribute to the reduced contraction in iron-deficient rats. The reason for the larger action potential amplitude in iron-deficient rats and its physiological implications are unclear.

Of the multiple ion currents underlying the action potential, we focused on \(I_{Ca,L}\), as it is the predominant current affecting contractile function and is a major target for the modulatory action of \(\beta\)-adrenergic stimulation. As shown by the \(I_{Ca,L}\) current–voltage (I-V) relations (Fig. 4) at a wide range of membrane potentials, \(I_{Ca,L}\) is smaller in myocytes from iron-deficient rats than from control rats (\(P < 0.05\)); this difference can certainly contribute to the reduced contractile force seen in this study. Next, we determined whether the increased \(\beta\)-inotropic responsiveness of iron-deficient rats was caused by an increased response of \(I_{Ca,L}\) to isoproterenol. Although isoproterenol increased \(I_{Ca,L}\) in both groups, the magnitude of the effect was not significantly different.
our previous studies on the effect of hypothyroidism on $I_{Ca,L}$ in guinea pig ventricular myocytes [22].

The reduced mechanical function in iron deficiency may have been caused by action potential shortening (Fig. 3), resulting from hypoxia and/or ischaemia (of the intact heart), which are frequently associated with anaemia [8, 25–28]. Hypoxia induced by metabolic inhibition (oxidative phosphorylation blockade) in guinea pig papillary muscle caused action potential shortening as a result of inhibition of $I_{Ca,L}$ and activation of $I_k$ [29]. Another possible explanation for action potential shortening in iron deficiency is increased transient outward current ($I_{to}$), which occurs in the ischaemic myocardium [30].

Another consequence of iron deficiency, possibly accounting for the reduced contraction, is the pronounced hypertrophy depicted by two indicators: increased heart/body ratio and increased myocyte length, width, circumference and area. In agreement with our findings, previous studies have reported decline in mechanical performance in the hypertrophied myocardium [8, 19, 27]. This decline was partly accounted for by the decreased concentrations of sarcoplasmic reticulum mRNA Ca$^{2+}$-ATPase and a shift from the V1 isoform to V3 [31]. The relative contribution of hypertrophy per se to the attenuated mechanical function in iron-deficient rats is presently unknown.

Owing to the central role of $\beta$-adrenergic receptors in modulation of cardiac function, we determined the effect of experimental iron deficiency on the inotropic responsiveness to isoproterenol. Of the twitch characteristics studied, the response to isoproterenol of twitch tension was enhanced by iron deficiency. We attempted to determine the mechanism for the enhanced responsiveness to isoproterenol in iron-deficient rats by testing the effect of isoproterenol on $I_{Ca,L}$ in both groups. The finding that $I_{Ca,L}$ responded similarly to isoproterenol in control and iron-deficient rats suggests that iron deficiency affects the excitation–contraction coupling pathway at a site distant to $I_{Ca,L}$. For example, iron deficiency may have caused increased responsiveness of the Ca$^{2+}$-induced Ca$^{2+}$-release mechanism and/or the contractile apparatus to Ca$^{2+}$.

In summary, experimental iron deficiency caused marked changes in cardiac mechanical and electrophysiological properties, as well as modified $\beta$-adrenergic responsiveness. The enhanced inotropic effect in experimental iron deficiency may have an important implication for iron-deficient anaemic patients: their reduced cardiac mechanical function may be compensated by an increased ability to respond to $\beta$-adrenergic stimulation.

ACKNOWLEDGMENTS

This study was supported by grants from the Israel Ministry of Health, Chief Scientist; by the Minerva Foundation through the Bernard Katz Center for Cell Biophysics; and by the Rappaport Family Institute for Research in the Medical Sciences.

REFERENCES

23. Reference deleted.
24. Reference deleted.


