Depressed contractile function and adrenergic responsiveness of cardiac myocytes in an experimental model of Parkinson disease, the MPTP-treated mouse

Jun Ren∗, James E. Porter, Loren E. Wold, Nicholas S. Aberle, II, Dhanasekaran Muralikrishnan, James R. Haselton

Department of Pharmacology, Physiology, and Therapeutics, University of North Dakota School of Medicine and Health Sciences, Grand Forks, ND 58203, USA

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Abstract

Radio tracer and biochemical studies have shown that patients with Parkinson disease lack functional sympathetic innervation to the heart. The same observation was made in mice treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), an experimental model of Parkinson disease. This study examined the mechanical properties, adrenergic receptor level and intracellular Ca2+ handling in cardiac myocytes isolated from C57BL/6 mice that received either MPTP (30 mg/kg, i.p., twice in 24 h) or vehicle. Mechanical properties were evaluated using an IonOptix MyoCam® system. Myocytes were electrically stimulated at 0.5 Hz. The contractile properties analyzed included peak shortening (PS), time-to-PS (TPS), time-to-90% relengthening (TR90), and maximal velocities of shortening and relengthening (±dL/dt). Intracellular Ca2+ handling was evaluated with fura 2. Myocytes from MPTP-treated mice exhibited a depressed PS (85% of normal), normal TPS, prolonged TR90 (147% of normal), and reduced ±dL/dt (both 79% of normal). These results were correlated with a 67% reduction of β3-adrenergic receptor expression in myocardial membranes from MPTP-treated mice when compared to normal. Myocytes from MPTP-treated mice also exhibited a reduced peak of intracellular Ca2+ sequestration and sarcoplasmic reticulum (SR) Ca2+ load (55 and 38% of normal, respectively). The resting intracellular Ca2+ and Ca2+-transient decay were comparable to the values seen in myocytes from untreated mice. Myocytes from MPTP-treated and untreated mice were equally responsive over a range of stimulation frequencies (0.1, 0.5, 1, 3 and 5 Hz). Response to norepinephrine (1 μM) and isoproterenol (1 μM) was reduced in myocytes from MPTP-treated mice. These results demonstrate substantial cardiac dysfunctions in this model of experimental Parkinson disease, probably due to reduced adrenergic responsiveness and SR Ca2+ load.

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1. Introduction

Numerous studies have demonstrated impaired sympathetic innervation of the heart in many patients with Parkinson disease [4,6,12,15,22,28,29,34]. Furthermore, the same impairment has been demonstrated in an experimental model of Parkinson disease, the mouse treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [28]. While the first report documenting sympathetic denervation of the heart in patients with Parkinson disease appeared in the literature in 1994, subsequent studies have not been undertaken to determine if there is an alteration of cardiac function in either experimental models of, or patients with, Parkinson disease. The impaired sympathetic innervation in Parkinson disease often results in the progression of interruption in sympathetic as well as parasympathetic control of cardiovascular function, ultimately leading to the deterioration of cardiovascular reflexes [11,29]. However, the direct impact of Parkinson disease on cardiovascular function has not been defined.

Neurotoxins such as MPTP and 6-hydroxy dopamine have been shown to cause dopamine depletion in the nigro-striatal tract of various animals. MPTP represents one of the most prominent neurotoxins that includes most, if not all, of the essential characteristics for a model of Parkinson disease. Monoamine oxidase-B is a mitochondrial enzyme that is
responsible for oxidative metabolism of MPTP to its ef-
fective toxic form, MPP+. The active dopaminergic toxin,
MPP+, is selectively taken up into the dopaminergic neuron
through the monoaminergic uptake system. Administra-
tion of high doses of MPTP to mice causes depletion of
dopamine in the nucleus caudate putamen. The toxicity is
mediated through a number of mechanisms, such as inhi-
bition of Complex-I of the mitochondrial electron transport
chain, generation of hydroxyl radicals leading to oxidative
stress and apoptosis [31].

To evaluate the direct effect of Parkinson disease on car-
diac contractile and adrenergic function, the present study
was conducted to determine if the mechanical function of
isolated ventricular myocytes is altered in an experimental
form of Parkinson disease, the MPTP-treated mouse.

2. Methods

2.1. Animals

All animal experimentation was conducted in accord with
humane animal care standards outlined in the NIH Guide
for the Care and Use of Experimental Animals. Adult male
C57 BL/6 mice (Jackson) were maintained on a 12:12 h
light–dark cycle, with free access to food and water. MPTP
hydrochloride (Research Biochemicals International, Nat-
wick, MA) was administered (30 mg/kg, i.p.) two times, 16 h
apart. This treatment regimen was used to deplete dopamine
in the substantia nigra [27], and has been shown to produce
a 75% reduction in dopamine concentration 7 days later
[31]. Normal mice received two injections of vehicle (0.9%
saline) 16 h apart. Mice were used for experiments at least 7
and not more than 14 days after MPTP treatment or vehicle
injection.

2.2. Cell isolation procedures

Hearts were rapidly removed from anesthetized mice and
immediately mounted on a temperature controlled (37 °C)
perfusion system. After perfusion with modified Tyrode
solution (CaCl2 free) for 2 min, the heart was digested
for 10 min with 0.9 mg/ml collagenase D (Boehringer
Mannheim Biochemicals) in modified Tyrode solution. The
modified Tyrode solution (pH 7.4) contained the following
(in mM): NaCl (135), KCl (4), CaCl2 (1), MgCl2 (1), glucose
(10), HEPES (10), at pH 7.4. The cells were field stimu-
lated with suprathreshold voltage at a frequency of 0.5 Hz,
3 ms duration, using a pair of platinum wires placed on op-
posite sides of the chamber connected to a FHC stimulator
(Brunswick, NE). The polarity of stimulation electrodes was
reversed frequently to avoid possible build up of electrolyte
by-products. The myocyte being studied was displayed on
the computer monitor using an IonOptix MyoCam camera,
which rapidly scans the image area at every 8.3 ms such that
the amplitude and velocity of shortening/relengthening is
recorded with good fidelity. The soft-edge software (IonOp-
tix) was used to capture changes in cell length during short-
ening and relengthening. All measurements were performed
at 25–27 °C.

2.4. Intracellular fluorescence measurement and
sarcoplasmic reticulum (SR) Ca2+ load

A separate cohort of myocytes was loaded with fura-2/AM
(0.5 μM) for 15 min and fluorescence measurements were
recorded with a dual-excitation fluorescence photomultiplier
system (Ionoptix) as previously described [20]. Myocytes
were placed on an Olympus IX-70 inverted microscope
equipped with a temperature-controlled (25 °C) Warner
chamber and imaged through a Fluor × 40 oil objective.
Cells were exposed to light emitted by a 75 W lamp and
passed through either a 360 or a 380 nm filter (bandwidths
were ±15 nm), while being stimulated to contract at 0.5 Hz.
Fluorescence emissions were detected between 480 and
520 nm by a photomultiplier tube after first illuminating
the 360 nm excitation scan was repeated at the end of the protocol
and qualitative changes in intracellular Ca2+ concentration
([Ca2+]i) were inferred from the ratio of the fluorescence
intensity at two wavelengths. The “interpolated” method
was used since 360 nm is the isobestic point for fura-2 at
which the numerator is independent of intracellular Ca2+ concentration.
The majority of the recording was done us-
ing the 380 nm filter because the strongest signal is the
380 nm-excited emission. Since intracellular Ca2+ tran-
sients in cardiac myocytes are short-lived with fast kinetics,
the “interpolated” method offers better signal-to-noise ratio
than the “interleaved” method (rapidly switching between
the 340 and 380 nm excitation wavelengths). Myocytes were discarded if the 360 signal displayed any decay >10%. The Chebyshev curve fitting technique was used to evaluate the intracellular Ca\textsuperscript{2+} decay kinetics. The goodness of fit was confirmed by visually inspecting the fitted curve to the actual data. Deviations were not accepted.

The SR load was directly assessed through a 10 s rapid application of caffeine (10 mM) with a rapid solution switcher to fura-2 loaded myocytes (0.5 \mu M for 10 min at 30 °C). The integration underneath the Ca\textsuperscript{2+} transient curve during the caffeine perfusion was calculated and used as an index of the SR Ca\textsuperscript{2+} load [21].

2.5. Membrane preparation for receptor binding

A crude membrane preparation was prepared as previously described [36]. Briefly, normal and treated C57 BL/6 mice were euthanized according to humane animal care standards, the heart rapidly excised and placed in 5 ml of cold buffer A containing 10 mM HEPES, 250 mM sucrose, 5 mM EGTA, 12.5 mM MgCl\textsubscript{2}, 10 \mu g/ml bacitracin, 10 \mu g/ml benzamidine, 10 \mu g/ml leupeptin, 20 \mu g/ml PMSF, 20 \mu g/ml soybean trypsin inhibitor, with a pH 7.5. The hearts were then Polytroned for 30 s and Dounced homogenized before being transferred to an equal volume of 0.5 M KCl. This mixture was stirred on ice for 30 min then homogenized again before being centrifuged at 1260 × g for 5 min at 4 °C. The supernatant was saved and centrifuged again at 30,000 × g for 15 min at 4 °C. This time the resultant pellet was kept, resuspended in buffer B (20 mM HEPES (pH 7.5), 100 mM KCl, 5 mM EGTA, 12.5 mM MgCl\textsubscript{2}) and recentrifuged at 30,000 × g for 15 min at 4 °C. The resulting crude membrane pellet was finally resuspended in buffer B containing 10% glycerol and stored in aliquots at −70 °C until used for radioligand binding. Protein concentrations were measured using the method of Bradford [2].

2.6. Radioligand binding

The radioligand binding protocol used for this study was performed as previously described [19]. Briefly, the density of expressed \beta\textsubscript{-}-adrenergic receptors on normal and MPTP-treated C57 BL/6 mice was determined by saturation binding experiments using the non-selective \beta\textsubscript{-}-adrenergic receptor antagonist (-)-3-[\textsuperscript{125}I]iodocyanopindolol (\textsuperscript{125}I-CYP), as the radiolabel (NEN Life Sciences). Crude myocyte membranes were allowed to equilibrate at 37 °C with increasing concentrations of \textsuperscript{125}I-CYP (5–600 pM) in a 0.25 ml total volume of buffer C (20 mM HEPES (pH 7.5), 100 mM KCl, 1.4 mM EGTA, 12.5 mM MgCl\textsubscript{2}) using 10\textsuperscript{−5} M propranolol to determine non-specific binding. Binding was stopped by filtering the membranes through Whatman GF/C glass fiber filters, followed by 5–5 ml washes with cold buffer C to remove any unbound drug. Amount of total and non-specific
membrane bound radiolabel were calculated from cpm counts remaining on the glass fiber filters. From the plotted saturation hyperbola, \( \beta \)-adrenergic receptor density and affinity value of \( ^{125} \text{I-CYP} \) were calculated using iterative non-linear regression analysis [17].

2.7. Statistical analyses

Nineteen normal and 19 MPTP mice were used in this study and the sample collected was distributed equally among all the animals. For each experimental series, data are presented as Mean \( \pm \) S.E.M. Statistical significance \((P < 0.05)\) for each variable was estimated by analysis of variance (ANOVA) or \( t \)-test, where appropriate (SYSTAT, Inc., Evanston, IL).

3. Results

3.1. Cell shortening and relengthening of cardiac myocytes from MPTP-treated and untreated mice

No obvious morphological alteration could be identified in cellular structure. MPTP treatment did not affect cell length (CL). The average resting CL of ventricular myocytes used in this study was 113 \( \pm \) 2 and 109 \( \pm \) 2 \( \mu \text{m} \) in the untreated and MPTP-treated groups \((n = 143–146 \text{ cells/group})\), respectively. Cells isolated from one heart were averaged and treated as one experiment. The peak shortening (PS) amplitude normalized to CL was significantly reduced in myocytes isolated from hearts of MPTP-treated mice. Myocytes from MPTP-treated mice also exhibited significantly prolonged time-to-90% relengthening (TR90), but time-to-PS (TPS) was comparable to that seen in the untreated group (Fig. 1). The reduced myocyte shortening and prolonged TR90 were associated with significantly reduced maximal velocities of both shortening \((+dL/dt)\) and relengthening \((-dL/dt)\) (Fig. 2).

3.2. Intracellular Ca\(^{2+}\) transients

We used the membrane permeable fura-2/AM to evaluate the properties of intracellular Ca\(^{2+}\) transients in myocytes from normal and MPTP-treated mice. The time course of the fluorescence signal decay was well described by a single exponential equation, and the time constant \((\tau)\) was used as a measure of the rate of decline of intracellular Ca\(^{2+}\). The fluorescence measurements revealed that the resting Ca\(^{2+}\) level was similar in both groups, whereas the increase of Ca\(^{2+}\) \((\Delta [\text{Ca}^{2+}]_{i})\) was significantly reduced in myocytes from the MPTP-treated group. The intracellular Ca\(^{2+}\) transients decay tended to be slower in myocytes from the MPTP-treated than in myocytes from the normal group; however, this was not significant (Fig. 3). These results revealed potential abnormalities in intracellular Ca\(^{2+}\) handling mechanisms in hearts from MPTP-treated mice. The traces in Fig. 3 were chosen to illustrate that MPTP depressed \( \Delta [\text{Ca}^{2+}]_{i} \). Myocyte shortening was also recorded from fura-2 loaded cells but these data were used for qualitative comparisons only, in order to avoid potential effects on contraction from intracellular Ca\(^{2+}\) buffering by fura-2.

3.3. SR Ca\(^{2+}\) load

SR Ca\(^{2+}\) load is the major pool of intracellular Ca\(^{2+}\) available to the contractile proteins during the excitation-contraction (E-C) coupling in cardiac myocytes. To evaluate the SR Ca\(^{2+}\) load in myocytes isolated from normal and MPTP-treated mice, myocytes were loaded with fura-2 for 10 min at 30 °C and were rapidly perfused with 10 mM caffeine for 10s [21]. The area underneath the intracellular Ca\(^{2+}\) transient curve was significantly smaller in myocytes from the MPTP-treated mice \((3248 \pm 1445 \text{ F*ms})\) compared to that of normal \((8633 \pm 1860 \text{ F*ms})\), \(n = 9–10 \text{ cells from five mice per group, } P < 0.05\) vs. normal group, indicating a reduced SR Ca\(^{2+}\) load in myocytes from MPTP-treated mice (Fig. 3).

3.4. Effect of stimulation frequency on myocyte shortening

Mouse hearts normally contract at very high frequencies (500 beats/min), whereas our baseline studies were...
conducted at 0.5 Hz. To look for possible derangement of cardiac E–C coupling at higher frequencies, we increased the stimulation frequency up to 5 Hz (300 beats/min) and recorded the steady-state shortening. Cells were initially stimulated to contract at 0.5 Hz for 5 min to ensure steady state before commencing the frequency sequence. Fig. 4 shows a similar pattern of negative staircase in PS with increasing stimulation frequency in both normal and MPTP groups. Although there is a trend of reduced PS value in myocytes from the MPTP group compared to normal ones, no statistical significance was reached. These data suggest that intracellular Ca\(^{2+}\) storage and release is likely preserved in MPTP mouse hearts. Note that the absence of a significant difference in PS between normal and MPTP groups at 0.5 Hz of stimulus frequency, where Fig. 1B suggests that there should be one, is likely due to the lower sample number required to test for the frequency response (103–104 cells from nine mice in Fig. 1B versus 13–14 cells from seven mice in Fig. 4).

3.5. Effect of norepinephrine and isoproterenol on myocyte shortening

To further explore the alteration in cardiac E–C coupling, myocytes were exposed to norepinephrine (NE, 1 \(\mu\)M) and isoproterenol (ISO, 0.1–1 \(\mu\)M) and myocyte shortening was examined. Myocytes from MPTP group exhibited a reduced responsiveness to both NE and ISO compared to that in the normal group (Fig. 5).

3.6. Receptor binding of cardiac membrane from MPTP-treated and untreated mice

To correlate the impaired myocyte mechanical function and reduced adrenergic responsiveness with \(\beta\)-adrenergic receptor expression, saturation binding analysis was performed on crude membranes isolated from the hearts of MPTP-treated and untreated mice (Fig. 6). Increasing amounts of \(^{125}\)I-CYP labeled an apparent homogeneous
Fig. 4. Peak shortening (PS) amplitude of ventricular myocyte isolated from normal and MPTP mouse hearts at different stimulus frequencies. Mean ± S.E.M., n = 13–14 cells from seven mice per group.

Population of binding sites in these membranes that was specific, saturable and showed high affinity. The radiolabel equilibrium dissociation constant (K_D) calculated from saturation isotherms of MPTP-treated membranes (50 ± 10 pM) was no different when compared to normal (60 ± 14 pM), indicating that MPTP did not affect the affinity of 125I-CYP for β-adrenergic receptors expressed on these myocardial membranes. However, there was a significant (P < 0.05) reduction of total β-adrenergic receptor expression on membranes isolated from MPTP-treated mice (76 ± 15 fmol/mg) when compared to untreated animals (227 ± 9 fmol/mg). This reduced receptor expression could potentially account for the impaired mechanical function and adrenergic responsiveness of myocytes isolated from MPTP-treated mice reported earlier in this study.

Fig. 5. Percent change from baseline cell shortening in myocytes isolated from normal and MPTP-treated mice when being exposed to norepinephrine (NE, 10^-6 M) or isoproterenol (ISO, 0.1–10^-6 M). Mean ± S.E.M., n = 13–26 cells from four to six mice/group, *P < 0.05 vs. baseline value, #P < 0.05 vs. normal group.

Fig. 6. Rosenthal plots constructed from the mean saturation binding data generated from mouse heart membranes treated (■) or untreated (□) with MPTP (six mice each). There were no differences in 125I-CYP affinities, represented by the regression line slope, for β-adrenergic receptor binding sites on these membranes. However, there was a significant (P < 0.05) reduction for β-adrenergic receptor expression, as indicated by the dissimilar X-intercept values, on MPTP-treated myocytes when compared to the normal ones.

4. Discussion

This study demonstrates mechanical dysfunction of cardiac myocytes in an experimental model (MPTP) of Parkinson disease. The major mechanical abnormalities observed in cardiac myocytes of MPTP-treated mice were decreased cell shortening, prolonged duration of relaxation, and reduced velocities of both shortening and relengthening. Decreased β-adrenergic receptor binding sites, SR Ca^2+ load and Δ[Ca^2+]i, may likely contribute, at least in part, to the impaired mechanical function in myocytes of MPTP-treated mice.

Several lines of evidence from anatomical studies have suggested that sympathetic innervation of the heart is altered in Parkinson disease. Lewy bodies, a hallmark of Parkinson disease, have been identified in both extrinsic and intrinsic sympathetic nerves of the heart in patients with Parkinson disease [14]. Radiotracers studies have documented that the sympathetic innervation of the heart is uniquely affected in Parkinson disease. These studies used the cardiac uptake of either 6-[18F]-fluorodopamine [11,12], or 123I-metaiodobenzylguanidine [3,4,6,10,13,15,18,22,26, 28-30,34,35] as a means of evaluating sympathetic function in the heart of patients with Parkinson disease. Both of these substances are processed by sympathetic nerve terminals in a manner that is identical to the processing of norepinephrine [5,9,12,16,23–25], and, therefore, their uptake was used as a non-invasive index of the integrity of sympathetic neurotransmission in the heart of patients with either Parkinson disease, or other parkinsonian diseases. The common finding reported in all of these studies is that the cardiac uptake of these substances is negligible, even in patients exhibiting the earliest stages of Parkinson disease. Furthermore, the
cardiac uptake of these substances is either not impaired, or minimally impaired in other parkinsonian diseases (e.g., pure autonomic failure [11], Shy-Drager syndrome [11], multiple system atrophy [4, 6, 18, 22, 29, 34], progressive supranuclear palsy [22, 29, 34], striatoniigral degeneration [34], and vascular parkinsonism [18, 22]). Indeed, several studies have suggested that the cardiac uptake of these substances might be useful for clinically differentiating Parkinson disease from other parkinsonian disorders [4, 6, 11, 18, 22, 29, 34].

A radiotracer study documented that cardiac sympathetic denervation also occurs in the MPTP-treated C57 BL/6J mouse [28], an experimental model of Parkinson disease [27]. In fact, cardiac sympathetic denervation was observed even in mice administered a very low dose (5 mg/kg) of MPTP [28], well below the dose that is used to induce parkinsonism in the same strain of mouse in this and other studies [27]. Other studies have shown that MPTP treatment produces depletion of cardiac catecholamines [1, 8, 33]. It is noteworthy that, after MPTP treatment, the highest concentration of 1-methyl-4-phenylpyridinium (MPP+) , the active toxin derived from MPTP, occurs in the heart [7]. These studies provide evidence of cardiac sympathetic denervation in both an experimental model of Parkinson disease as well as the actual disease, but do not address the possibility of a functional impairment in the sympathetic control of the heart in either of these conditions. The present study demonstrated impairment of the mechanical functioning of the heart, intracellular calcium handling, and responsiveness to norepinephrine in experimental Parkinson disease.

The most prominent cardiac dysfunctions in the MPTP-treated mice observed in the current study were reduced cardiac contraction (PS), and prolonged relaxation duration (TR90), associated with diminished maximal velocity of contraction/relaxation (±dL/dt). Several factors may have contributed to the impaired mechanical functions including defective function of contractile proteins (e.g. actin, myosin heavy chain isoform), reduced intracellular Ca^{2+} availability due to depressed SR Ca^{2+} load, or altered myofilament Ca^{2+} sensitivity. The intracellular Ca^{2+} measurement confirmed a decrease in intracellular Ca^{2+} recruitment in response to electrical excitation in myocytes from MPTP-treated mice, although other possibilities may exist but remain to be identified. The observation that intracellular Ca^{2+} decay was comparable between the normal and MPTP mouse heart indicates that the prolonged TR90 was probably not due to reduced intracellular Ca^{2+} clearing from sarcoplasmic/endoplasmic Ca^{2+}-ATPase and Na^{+}/Ca^{2+} exchanger. On the other hand, in this study the steady-state myocyte shortening in response to increases in stimulating frequency was unaffected by MPTP treatment, which suggests that intracellular Ca^{2+} storage and clearing were preserved after MPTP treatment.

Catecholamine desensitization is considered one of the hallmarks of heart failure. Heart failure has been shown to induce classic catecholamine desensitization to inotropic responses by both isoproterenol and norepinephrine in the presence and absence of ganglionic blockade [32]. This is similar to our observation of the reduced responsiveness to norepinephrine and isoproterenol in the MPTP mouse myocytes. Our receptor binding study further revealed that the number of β-adrenergic receptors but not their affinity is likely to contribute to the reduced adrenergic responsiveness and ultimately, impaired cardiac mechanical function in MPTP-treated mice. Although it appears that sympathetic denervation may lead to postsynaptic supersensitivity due to lack of neural uptake for norepinephrine [32], the extent to which different models of denervation demonstrate supersensitivity remains controversial.

In view of the fact that studies of patients with Parkinson disease have sympathetic cardiac denervation that is comparable to that seen in the MPTP-treated mouse [28], our results suggest that cardiac function may be impaired in patients with this disease. This possibility is also supported by the results of Goldstein et al. [12], demonstrating that noradrenergic neurotransmission to the heart is impaired. They observed profound deficits in cardiac extraction of [3H]-norepinephrine, cardiac norepinephrine spillover, and venous-arterial increments in plasma levels of DHPG and t-dopa in all six of the Parkinson disease patients that they examined. No data have been reported about alterations in the regulation of β-adrenergic receptors in either patients with Parkinson disease, or in the MPTP-treated mouse.

In summary, this study provides the first evidence of altered cardiac contractile function at the ventricular myocyte level in an experimental model of Parkinson disease. Myocytes from MPTP-treated mice exhibited decreased inotropic response, lusitropic response, and intracellular Ca^{2+} sequestration, probably due to mechanisms associated with reduced SR Ca^{2+} load and reduced adrenergic function. However, other mechanisms cannot be excluded at this time such as susceptibility of myofilaments to Ca^{2+} and alteration of myosin heavy chain isoform. Future studies will (1) elucidate the intracellular signaling mechanisms that are responsible for these effects, and (2) identify the causal relationship between loss of sympathetic innervation and mechanical dysfunction of the heart in this model of Parkinson disease.

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