Short-Term Effects of Prednisolone on Neuromuscular Transmission in the Isolated Mdx Mouse Diaphragm

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\(^{1}\)Department of Neurology, Kwavatana National Hospital, Nagasaki 859–3615, \(^{1}\)School of Allied Medical Science, and \(^{2}\)The First Department of Internal Medicine, Nagasaki University, Nagasaki 852–8501

Fukudome, T., Shibuya, N., Yoshimura, T. and Eguchi, K. Short-Term Effects of Prednisolone on Neuromuscular Transmission in the Isolated Mdx Mouse Diaphragm. Tohoku J. Exp. Med., 2000, 192 (3), 211–217 — To determine the mechanism of the beneficial effects of prednisolone on Duchenne muscular dystrophy (DMD), we examined the short-term effects of prednisolone on neuromuscular transmission by using conventional microelectrode methods in the mdx mice. High (56 µmol/liter) and low (2.8 µmol/liter) concentrations of prednisolone were applied to a bath containing phrenic nerve-diaphragm preparations from mdx mice, and several parameters related to neuromuscular transmission were recorded. The high dose of prednisolone significantly decreased parameter \(n\) on quantal release by nerve impulse and decay time-constant of end-plate potentials, which showed adverse effect on neuromuscular transmission. The low dose of prednisolone did not significantly increase quantal content, but could assist the compensatory reaction to maintain the safety margin of neuromuscular transmission in the mdx mice. Our results suggest that the latter effect represents one of the possible mechanisms of the therapeutic effects of prednisolone on DMD. ——— Duchenne muscular dystrophy; prednisolone; mdx mice; microelectrode study; neuromuscular transmission \(©\) 2000 Tohoku University Medical Press

Previous studies have shown that prednisolone can improve muscle strength in Duchenne muscular dystrophy (DMD) (Drachman et al. 1974; Brooke et al. 1987; DeSilva et al. 1987; Mendell et al. 1989; Fenichel et al. 1991) probably by increasing dystrophin expression (Hardiman et al. 1993) or by reducing phospholipase A2 activity (Flower 1990), although the primary mechanism remains unknown. The mdx mouse, an animal model of DMD, shows similar ultrastructural abnormalities at the neuromuscular junction to those noted in patients with DMD.

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Electrophysiological studies have demonstrated a low amplitude of miniature end-plate potential (MEPP) in mdx mice, which is probably due to the ultrastructural changes at the neuromuscular junction as well as the associated increase in the quantal content of the end-plate potential to maintain a safety margin of neuromuscular transmission (Nagel et al. 1990). At concentrations ranging from 4 to 16 $\mu$mol/liter, prednisolone exhibits a presynaptic effect at the neuromuscular junction and increases MEPP amplitude in the isolated rat diaphragm (von Wilgenburg 1979). Prednisolone may produce its beneficial effect in human DMD and mdx mice by maintaining the safety margin of neuromuscular transmission.

In the present study, we investigated the effects of bath application of low (2.8 $\mu$mol/liter) and high (56 $\mu$mol/liter) concentrations of prednisolone on neuromuscular transmission using an isolated mdx mouse diaphragm muscle. To know the short-term effects in the different concentration may be useful for supposing the long-term effects or safety of prednisolone in human DMD.

**Materials and Methods**

**Animals**

Animal experiments were conducted under the Guidelines of the Animal Care and Use Committee of Nagasaki University. Six female mdx mice aged 8 to 10 weeks were used in all experiments.

**Electrophysiological studies**

For conventional intracellular microelectrode recording, a portion of the mouse diaphragm with its motor nerve was dissected carefully and removed. The phrenic nerve-diaphragm muscle preparation was mounted in a chamber perfused continuously with Tyrode solution containing (mM): KCl, 5; Na$_2$HPO$_4$, 1.3; MgCl$_2$, 1; NaCl, 135; CaCl$_2$, 2; and glucose, 11.1; at pH 7.2. The perfusate was continuously bubbled with 95% O$_2$-5% CO$_2$ gas mixture. For intracellular recording, the preparation was first perfused with prednisolone (Shionogi Inc., Tokyo) at a concentration of 1 mg/liter (2.8 $\mu$mol/liter) or 20 mg/liter (56 $\mu$mol/liter) for 45 minutes or longer at the recording temperature and the same agents were also added to the perfusate during recording. d-Tubocurarine chloride was used at a concentration sufficient to inhibit muscle contraction. MEPPs, end-plate potentials (EPPs) and resting membrane potentials (RMPs) were recorded while the nerve-muscle preparation was perfused with Tyrode solution at 29.5 ± 0.5°C. Recordings were obtained using 13–18 MΩ glass microelectrodes and MEZ-8300 amplifier (Nihon Kohden, Tokyo). Signals were digitised at 10 kHz through Digidata 1200B (Axon Instruments, Foster City, CA, USA) interface, stored on a hard disk and analysed by Axoscope 1.1 (Axon Instruments). Analysis of MEPPs and EPPs was restricted to potentials with a rise time < 1.5 milliseconds and to fibres with resting membrane potential of $<-60$ mV. Potentials were corrected to a standard resting membrane potential of $-80$ mV.
assuming an equilibrium potential of 0 mV (Cull-Candy et al. 1979). MEPPs with more than twice the mean amplitude were rejected as "giant MEPPs."

For EPP recording, the phrenic nerve was stimulated using a suction electrode at 0.5 Hz. The potentials were corrected for non-linear summation (Martin 1966) and the last 64 responses in a train of 114 were saved for later analysis. The quantal content, m, was calculated by the variance method (Elmqvist and Quastel 1965). The latter was based on a Poisson distribution of m, but when m is greater than 8, it deviates increasingly from a Poisson distribution, and m values were corrected for a non-Poisson release process by the empiric formula \( m_{\text{corr}} = 1.743 m^{0.7328} \) (Cull-Candy et al. 1980). Estimates of the binomial release parameters \( p \), the probability of release, and \( n \), the number of quantal units capable of responding to nerve impulses (del Castillo and Katz 1954), were obtained by plotting the quantal content of each of the first four EPP in a train at 40 Hz against the sum of the quanta released by the preceding stimuli (Elmqvist and Quastel 1965). In some motor end-plates, 40 Hz stimulation was repeated three times at intervals of 60 seconds and results were averaged.

The decay time-constant of the EPP was derived by the fit of exponential functions to the decay phase of the EPP calculated by the computer. For each animal, MEPPs or EPPs were recorded from at least 15 or 10 different end-plates, respectively, before exposure to prednisolone.

Statistical analysis

All data are expressed as mean±standard error of the mean (s.e.m.). Differences between groups were examined for statistical significance using the Student’s t-test. A p-value < 0.05 denoted the presence of a significant statistical difference.

Results

Effects of prednisolone on miniature end-plate potentials and end-plate potentials

The effect of prednisolone was tested at concentrations of 1 mg/liter and 20 mg/liter. None of these concentrations had a significant effect on the frequency or resting membrane potential. However, prednisolone at 1 mg/liter but not at 20 mg/liter, significantly increased the amplitude of MEPPs (p < 0.05, Table 1). At

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Amplitude (mV)</th>
<th>Frequency (min(^{-1}))</th>
<th>RMP (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (50)</td>
<td>1.21 ± 0.08</td>
<td>32 ± 2.4</td>
<td>-69 ± 0.5</td>
</tr>
<tr>
<td>1 mg/liter prednisolone (48)</td>
<td>1.55 ± 0.10*</td>
<td>33 ± 2.4</td>
<td>-69 ± 0.5</td>
</tr>
<tr>
<td>20 mg/liter prednisolone (45)</td>
<td>1.41 ± 0.08</td>
<td>30 ± 2.4</td>
<td>-68 ± 0.5</td>
</tr>
</tbody>
</table>

Values are mean±s.e.m. RMP, resting membrane potential.

*\( p < 0.05 \). Numbers of end-plates are given in parentheses.
Table 2. Effects of prednisolone on quantal release by nerve impulse

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Quantal content (m)</th>
<th>p</th>
<th>n</th>
<th>$\tau_{EPP}$(milliseconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>34.8 ± 1.5 (39)</td>
<td>0.068 ± 0.0041 (18)</td>
<td>660 ± 49 (18)</td>
<td>2.4 ± 0.11 (39)</td>
</tr>
<tr>
<td>1 mg/liter prednisolone</td>
<td>36.8 ± 1.8 (37)</td>
<td>0.12 ± 0.013* (21)</td>
<td>460 ± 51* (21)</td>
<td>2.4 ± 0.17 (37)</td>
</tr>
<tr>
<td>20 mg/liter prednisolone</td>
<td>31.6 ± 1.8 (34)</td>
<td>0.073 ± 0.022 (15)</td>
<td>450 ± 140* (15)</td>
<td>1.7 ± 0.19* (34)</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M.

$^1\tau_{EPP}$ indicates the decay time-constant of EPP.

* $p < 0.05$. Numbers of end-plates are given in parentheses.

Fig. 1. Effect of prednisolone on EPP. Each panel represents the averaged trace of 64 EPPs and the exponential fitting to the decay phase of EPP. Without prednisolone (control), the decay time-constant of EPP ($\tau_{EPP}$) was $2.4 \pm 0.11$ milliseconds. During application of 1 and 20 mg/liter prednisolone, the $\tau_{EPP}$ was $2.4 \pm 0.17$ and $1.7 \pm 0.19$ milliseconds, respectively.
20 mg/liter, prednisolone significantly reduced the decay time-constant of EPP ($\tau_{\text{EPP}}$) by 29% ($p < 0.05$, Table 2 and Fig. 1). All observed EPPs decayed mono-exponentially.

**Effects of prednisolone on quantal release by nerve impulse**

The quantal content of the EPP ($m$) increased during application of 1 mg/liter prednisolone but decreased during application of 20 mg/liter, although none of these changes were significant. Parameter $p$ increased significantly (by 70%) during application of 1 mg/liter prednisolone, but not at 20 mg/liter. Parameter $n$ diminished by 23–38% and by 10–53% at 1 and 20 mg/liter prednisolone, respectively.

**DISCUSSION**

The peak serum concentrations of prednisolone after a high-dose intravenous infusion (26 mg/kg) and low-dose oral intake (0.8 mg/kg) ranges between 18–22 mg/liter (Ito et al. 1992) and 0.6–0.7 mg/liter, respectively. Prednisolone concentration used in our study; 20 mg/liter and 1 mg/liter were clinically attainable during therapy.

Our results showed that at 1 mg/liter, prednisolone increased the amplitude of MEPPs, $m$, and parameter $p$ but decreased parameter $n$ in quantal release by nerve impulse. Although the increment of $m$ was not significant, the amplitude of MEPPs increased significantly and the increment of parameter $p$ (76%) was greater than the decrement of $n$ (30%), indicating that 1 mg/liter prednisolone increases $m$. On the other hand, 20 mg/liter prednisolone did not affect any parameter of MEPPs, or $p$ but decreased $m$ and $n$. The decrement of $m$ was not significant, but $n$ was significantly lower than the control. Furthermore, the decay time-constant of EPP diminished significantly at 20 mg/liter prednisolone.

The glucocorticoid hydrocortisone is known to influence acetylcholine receptor channel as a long-term open channel blocker (Bouzat and Barrentes 1996). This action can explain the decrease in decay time-constant of EPP observed in our study during application of prednisolone. The decay time-constant represents the mean duration of opening of the acetylcholine receptor channel. In this regard, a reduction in channel open time compromises the safety margin of neuromuscular transmission. Thus, the effect of 20 mg/liter prednisolone is mediated post-synaptically (blocks acetylcholine receptor) more than presynaptically (reduces quantal content), with the resultant adverse effects on neuromuscular transmission.

In comparison with age-matched control mice, the mdx mice show an abnormal, age-dependent decrease in the amplitude of the miniature end-plate potential and a concomitant increase in the quantal content of the end-plate potential (Nagel et al. 1990). Increased quantal content could be a compensatory reaction to offset the fall in MEPP amplitude and thereby maintain the safety margin of
neuromuscular transmission (Nagel et al. 1990). Our results suggest that 1 mg/liter prednisolone does not cause a significant increase in quantal content, but could assist the compensatory reaction to maintain the safety margin of neuromuscular transmission during short-term treatment.

Treatment with prednisolone at 2 mg/kg/day for 8 weeks produces beneficial effects in the mdx mouse by protecting against muscle weakness (Hudecki et al. 1993) and at higher dose (5 mg/kg/day for 8 days), prednisolone protects against muscle damage during exercise in rats (Jacobs et al. 1996). In mdx mice, the safety margin of neuromuscular transmission is not impaired as determined by conventional microelectrode studies (Nagel et al. 1990), but these mice show significant fatigue on variable-speed treadmill exercise (Hudecki et al. 1993).

Our results suggest that short-term treatment with low-dose prednisolone could assist in maintaining neuromuscular transmission and it may protect against neuromuscular transmission failure during exercise. This is one of the possible mechanisms to prevent fatigue during exercise. Since it has been postulated that the abnormal dystrophic fibers may be susceptible to damage caused by contraction and stretch (Weller et al. 1990), protecting against fatigue may be beneficial both for mdx and DMD muscle. Further study is needed about the effects of long-term treatment, this mechanism may have a beneficial potential to protect against muscle damage during exercise.

In the mdx mouse, the short-term treatment with high dose prednisolone compromised the neuromuscular transmission. In case of the high-dose intravenous infusion therapy for DMD patients, careful observation might be needed to prevent the fatal neuromuscular insufficiency, such as respiratory failure.

References

8) Elmqvist, D. & Quastel, D.N.J. (1965) A quantitative study of end-plate potentials


