THE NOVEL TrkB RECEPTOR AGONIST 7,8-DIHYDROXYFLAVONE ENHANCES NEUROMUSCULAR TRANSMISSION

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ABSTRACT: Neurotrophin signaling at the neuromuscular junction modulates cholinergic transmission and enhances neuromuscular transmission via the tropomyosin-related kinase receptor subtype B (TrkB). A novel flavonoid, 7,8-dihydroxyflavone (7,8-DHF), selectively activates TrkB receptors. Using TrkBF616A mice that are susceptible to specific inhibition of TrkB activity by 1NMPP1, we show that neuromuscular transmission is enhanced by 7,8-DHF (~32%) via activation of TrkB in diaphragm muscle. The small molecule 7,8-DHF may constitute a novel therapy to improve neuromuscular function.

METHODS

Several studies have demonstrated that neurotrophin signaling at the neuromuscular junction modulates cholinergic transmission.1–5 In particular, brain-derived neurotrophic factor (BDNF) potentiates neurotransmitter release in both developing neuromuscular synapses in culture,3,4,6 and at adult rat neuromuscular junctions.1,2,5 BDNF binds the tropomyosin-related kinase receptor subtype B (TrkB) with high affinity to exert its biological effects.7,8 One member of the flavonoid family of chemicals, 7,8-dihydroxyflavone (7,8-DHF), was recently reported to selectively activate TrkB,9,10 although some neuroprotective effects of 7,8-DHF may not be mediated via TrkB activation.11 The purpose of this study was to determine the effect of 7,8-DHF on neuromuscular transmission using TrkBF616A mice that are susceptible to specific inhibition of TrkB activity by 1NMPP1.12 Administration of neurotrophins like BDNF has proven clinically unfeasible,13–15 but therapeutic limitations may be overcome by small-molecule TrkB receptor agonists such as 7,8-DHF.

RESULTS

Diaphragm muscle force decreased with repetitive stimulation at 40 Hz, as previously reported in rats.1,16–19 Diaphragm muscle contractile and fatigue properties were not different across treatment groups. Specifically, twitch force generated by the diaphragm muscle was 7.9 ± 0.6 N/cm² for vehicle-treated, 7.4 ± 0.3 N/cm² for 7,8-DHF-treated, and 7.5 ± 0.2 N/cm² for 1NMPP1- and 7,8-DHF-treated groups. Diaphragm muscle tetanic

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A small-molecule member of the flavonoid family of chemicals, 7,8-DHF, rapidly enhanced neuromuscular transmission in the diaphragm muscle of adult TrkBFl616A mice. This improvement was produced by specific activation of the TrkB receptor, because inhibition of TrkB activity by 1NMPP1 abrogated this effect. TrkBFl616A mice were genetically modified to stably harbor a knock-in mutation in the exon encoding the Trk kinase ATP-binding pocket that renders TrkB susceptible to inhibition by derivatives of the kinase inhibitor PP1.12

The compound 7,8-DHF likely enhances neuromuscular transmission presynaptically, because it does not directly affect isometric contractile and fatigue properties of the diaphragm muscle. The effects of 7,8-DHF are consistent with the role of neurotrophins on neuromuscular transmission.2–4,6

In rats, neuromuscular transmission is enhanced by treatment with the neurotrophins BDNF and NT-4 (−31%),1 both of which bind the TrkB receptor.7,8 Neither BDNF nor NT-4 treatment affected muscle contractile or fatigue properties in rats.

TrkB receptor activity may help sustain neuromuscular transmission during repetitive stimulation. Indeed, concomitant treatment with 1NMPP1 (and 7,8-DHF) worsened neuromuscular transmission compared with vehicle. In the rat diaphragm muscle, treatment with K252a, a TrkB receptor inhibitor, also impaired neuromuscular

observed (P < 0.001). Compared with vehicle controls, 7,8-DHF treatment reduced the contribution of neuromuscular transmission failure to muscle fatigue after 75 s of repetitive 40-Hz stimulation (P < 0.001), indicating an enhancement of neuromuscular transmission with 7,8-DHF treatment. Concomitant treatment with 1NMPP1 completely abolished the effect of 7,8-DHF and, after 105 s of repetitive stimulation, neuromuscular transmission was impaired compared with vehicle-treated controls (P < 0.001). After 2 min of repetitive nerve stimulation, the contribution of neuromuscular transmission failure to diaphragm muscle fatigue was 37.7 ± 1.7% for the vehicle-treated group, 25.6 ± 3.3% for the 7,8-DHF-treated group, and 52.8 ± 3.4% for the 1NMPP1- and 7,8-DHF-treated group (P < 0.05 for both of the 7,8-DHF-treated groups compared with vehicle, and for the 1NMPP1-treated vs. 7,8-DHF alone groups). In summary, 7,8-DHF treatment enhanced neuromuscular transmission by −32%, whereas concomitant treatment with 1NMPP1 impaired neuromuscular transmission by −40%, indicating that 7,8-DHF rapidly potentiates neuromuscular transmission via activation of TrkB receptors.

DISCUSSION

A small-molecule member of the flavonoid family of chemicals, 7,8-DHF, rapidly enhanced neuromuscular transmission in diaphragm muscle of TrkBFl616A knock-in mice. This improvement was produced by specific activation of the TrkB receptor, because inhibition of TrkB activity by 1NMPP1 abrogated this effect. TrkBFl616A mice were genetically modified to stably harbor a knock-in mutation in the exon encoding the Trk kinase ATP-binding pocket that renders TrkB susceptible to inhibition by derivatives of the kinase inhibitor PP1.12

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force was 21.4 ± 0.4 N/cm², 21.0 ± 0.5 N/cm², and 21.8 ± 0.6 N/cm² in the vehicle-, 7,8-DHF-, and 1NMPP1-treated groups, respectively. Diaphragm muscle fatigability assessed as the decrement in force generated after 2 min of repetitive 40-Hz stimulation was also not different across groups (−35% of initial force). Thus, TrkB receptor signaling at the neuromuscular junction did not affect the muscle fiber properties in these mice, which is consistent with a previous report in rats.1

Neuromuscular transmission failure was estimated by comparing the forces generated by nerve and direct muscle stimulation (Fig. 1). A significant experimental group and time effect was

FIGURE 1. Effect of 7,8-dihydroxyflavone on neuromuscular transmission in diaphragm muscle of TrkBFl616A knock-in mice. (A) Representative tracing of force evoked by repetitive stimulation of a diaphragm–phrenic nerve preparation. Maximal isometric twitch force was obtained by direct muscle stimulation (first peak). The phrenic nerve was then stimulated at 40 Hz. Direct muscle stimulation was superimposed every 15 s via plate electrodes. Repetitive stimulation results in muscle fatigue, which is evident as a decrease in force. The contribution of neuromuscular transmission failure to muscle fatigue can then be calculated from the difference in force elicited by nerve and muscle stimulation (see text for details). (B) Time course of neuromuscular transmission failure over time, which is consistent with previous results in the rat.1,16–19 Treatment with 7,8-dihydroxyflavone (filled circles) reduced neuromuscular transmission failure, reflecting enhanced neuromuscular transmission. The effect of 7,8-dihydroxyflavone was completely blocked by 1NMPP1 treatment (open triangles), indicating a specific effect on TrkB receptor kinase activity, because TrkBFl616A knock-in mice harbor a mutation that renders TrkB susceptible to inhibition by 1NMPP1.12 *Different from vehicle-treated group at the same time-point. #Different from 1NMPP1- and 7,8-dihydroxyflavone–treated group at same time-point (P < 0.05).
transmission. Furthermore, both K252a and the fusion protein TrkB-IgG, which binds endogenously released BDNF or NT-4, reduced cholinergic release in the mouse levator auris longus muscle. Of note, 7,8-DHF exerted neuroprotective effects in the hippocampus that were mediated by TrkB receptor signaling. However, 7,8-DHF prevented apoptosis of cultured hippocampal cells lacking TrkB receptor, and these effects were mediated by reduced generation of reactive oxygen species (ROS). In the frog neuromuscular junction, ROS inhibit transmitter release, but it is presently unknown whether ROS exert a similar effect in rodents. Regardless, in this study, concomitant treatment with 1NMPP1 in TrkB F616A mice completely blocked the effect of 7,8-DHF.

Treatment with 7,8-DHF may yield the beneficial effects of neurotrophins, but without the side-effect profile and bioavailability issues that have limited human application. Enhanced neuromuscular transmission by pharmacological agents such as 7,8-DHF may constitute a novel therapy for neuromuscular disorders, but the clinical impact of such therapy remains to be determined.

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REFERENCES

CONCENTRIC NEEDLE JITTER IN STIMULATED FRONTALIS IN 20 HEALTHY SUBJECTS

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ABSTRACT: Normative data for jitter parameters using a disposable concentric needle have been presented in a few studies. Jitter, expressed as the mean consecutive difference (MCD), was measured in the frontalis muscle in 20 subjects by percutaneous bar stimulation of the temporal nerve branch. The mean MCD for individual studies (20) and for all potentials (600) were 16.05 ±

Abbreviations: ASFAP, apparent single-fiber action potential; CNE, concentric needle electrode; FR, frontalis muscle; MCD, mean consecutive difference; SFAP, single-fiber action potential; SFEMG, single-fiber electromyography, stimulation jitter

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2.73 µs and 16.05 ± 5.96 µs, respectively. The suggested limit for mean MCD is 22 µs and for outliers is 28 µs.


Single-fiber electromyography (SFEMG) measures neuromuscular jitter, which represents the variation in time intervals between pairs of single-fiber action potentials (SFAPs) obtained with voluntary activation or the variation in time measured between stimulus and evoked SFAPs in response to nerve stimulation (s-jitter). Jitter is a sensitive measurement of neuromuscular junction function and


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is most valuable clinically in evaluation of patients with suspected myasthenia gravis.

Due to the increasing concern for the transmission of infections, disposable concentric needle electrodes (CNE) are being used for jitter analysis, instead of the reusable and expensive SFEMG needle electrodes.

For CNE jitter measurements, the low-frequency filter should be raised from 500 Hz to 1 kHz to suppress activity from distant muscle fibers. As the signals obtained with CNE recording do not always represent a SFAP, but rather a summation of many, the term "jitter recording with CNE" is more accurate than SFEMG. The term "apparent single-fiber action potentials" (ASFAPs) is preferred instead of SFAPs.

METHODS

In this study, we prospectively studied 20 healthy subjects (6 men and 14 women) with a mean age of 38 ± 10.8 years (range 20–57 years). None had been diagnosed with a neuromuscular disorder, an unrelated medical condition, or were taking medications that could have interfered with the study, such as calcium channel blockers. The study was approved by the ethics committee of the Faculdade de Medicina de São José do Rio Preto, and informed consent was obtained from each subject.

A portable KeypointNet electromyograph (Medtronic Skovlunde, Denmark) with built-in jitter software was used for recording and analysis, using a peak detection algorithm for time measurements. The recordings were performed using a CNE with a diameter of 0.30 mm and a recording area of 0.019 mm² (Alpine bioMed, Denmark).

The temporal branch of the facial nerve was stimulated with a bar electrode (percutaneous), about 2 cm lateral and 2 cm above from the canthus (Fig. 1). Stimulation frequency was set at 10 Hz. The stimuli were delivered as rectangular pulses of 0.10 ms duration, and the intensity was adjusted to produce a slight visible twitch of the frontalis (FR) muscle. In general, this could be achieved at about 4–7 mA. The CNE was inserted in the middle of the forehead and positioned to record clearly defined spike components. The jitter was measured between stimulus and spike when a further increase in stimulus intensity no longer decreased the jitter for the components to be studied according to standard methods. ASFAPs should have a fast rising phase without notches or shoulders and have a well-defined peak. The shape should be constant at consecutive discharges and is best seen when 5–10 traces are superimposed and inspected at a sweep speed of 0.5 ms/division. The negative-going deflection of the waveforms should be parallel on superimposed traces.

Jitter was expressed as the mean of mean consecutive differences (MCD) in 30 analyzed potentials. Filter settings were 1 kHz to 10 kHz. MCD parameters were assessed by obtaining mean and standard deviation (SD) for Gaussian distributions or median and percentiles for non-Gaussian distributions. The upper limit of normality was set at the 97.5th percentile for non-Gaussian distributions and the mean plus 2 SDs for Gaussian distributions.

RESULTS

The mean jitter was analyzed according to a previously used method, i.e., a calculation of the mean MCD of 30 isolated potentials for s-jitter. The mean of 20 MCD values in each subject was 16.05 ± 2.73 μs and ranged from 11.2 to 20.5 μs with a Gaussian distribution. The upper 97.5% confidence limit was 21.51 μs (+2 SD). The mean of all 600 ASFAP MCD values was 16.05 ± 5.96 μs, ranging from 6.1 to 39.1 μs with a non-Gaussian distribution. The upper 97.5% confidence limit was 27.73 μs.

DISCUSSION

In this study we measured the jitter values obtained after facial temporal branch stimulation in the FR muscle. For CNE jitter, a few laboratories have reported reference values. Often, the spikes obtained with CNE are not obtained from single muscle fibers, but represent summation of more than one SFAP. Therefore, separate normative data from those published for SFEMG should be collected for CNE recordings.

The jitter reference values described using SFEMG electrode and needle stimulation in the FR were 14.7 ± 2.8 μs for MCD and 14.6 ± 6.8 μs for all SFAPs; in another study these values were 15 ± 3 μs for MCD and 15 ± 7 μs for all SFAPs. Their results are similar to ours.

In all recordings we ascertained that the stimulus strength was supramaximal, i.e., a further increase
in stimulus strength did not further decrease the jitter generated at the stimulus site. Comparison of jitter values for percutaneous and near-nerve needle stimulation for orbicularis oculi and frontalis and for orbicularis oculi alone showed no difference.

Peak detection rather than amplitude level for the time markers seems better for ASFAPs because of less influence from summation. With riding signals, the jitter value is erroneously higher when the amplitude level trigger method is used.

This study has defined reference limits obtained from 20 healthy subjects. For an individual patient we suggest using a mean MCD upper limit of 22 μs and a limit of 28 μs for individual recordings (outlier values). CNE is an acceptable alternative to SFEMG for acquiring ASFAPs after percutaneous nerve stimulation. It is safe, easy, quick, well supported, and reliable. Still, a certain level of caution must be maintained before a study using CNE is declared abnormal or normal in situations of borderline jitter values until a consensus is reached on definitions of acceptable recordings, filter settings, and analysis methods. After these definitions are established, a larger reference population should be studied in a multicenter investigation.

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