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ORIGINAL ARTICLE

Octanoic Acid Suppresses Harmaline-Induced Tremor in Mouse Model of Essential Tremor

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Abstract Recent work exploring the use of high-molecular weight alcohols to treat essential tremor (ET) has identified octanoic acid as a potential novel tremor-suppressing agent. We used an established harmaline-based mouse model of ET to compare tremor suppression by 1-octanol and octanoic acid. The dose-related effect on digitized motion power within the tremor bandwidth as a fraction of overall motion power was analyzed. Both 1-octanol and octanoic acid provided significant reductions in harmaline tremor. An 8-carbon alkyl alcohol and carboxylic acid each suppress tremor in a pre-clinical mouse model of ET. Further studies are warranted to determine the safety and efficacy of such agents in humans with ET.

Keywords 1-octanol · Octanoic acid · Essential tremor · Harmaline · Therapeutics

Introduction

Postural and kinetic tremors are the primary manifestations of essential tremor (ET). The physiological basis for these disabling tremors is not well understood, although the inferior olive and cerebellum have been implicated as tremor generators [1], along with reductions in gamma-aminobutyric acid (GABA) receptor concentrations within the dentate nucleus of postmortem ET brains [2] and reduced GABAergic function using ^{11}C-flumazenil positron emission tomograpy [3]. Current treatment options for ET are limited and provide only partial reductions in tremor. Only 2 drugs (primidone and propranolol) have been given a level A recommendation for efficacy by the American Academy of Neurology [4]. Side effects are common, however, and often lead to patient noncompliance. Thalamic deep brain

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stimulation (DBS) is an option for severely disabled patients. While usually highly effective, DBS has various risks, high cost and the chance of gradual tremor return [5]. Patients note that ethanol reduces their tremor, with ≤74% reporting a response [6]. Unfortunately, the use of ethanol is not practical for multiple reasons, including potential for intoxication, abuse, and rebound tremor [7].

In contrast to ethanol (C_2H_5-OH), whose tremor suppression effects approach intoxication in some patients, higher molecular weight alcohols (C_8H_{17}-OH and C_9H_{19}-OH) offer tremor suppression with much less risk of intoxication. Through intracellular recording, 1-octanol has been shown to block inferior olivary low threshold calcium channels [8], which have been linked to synchronized oscillatory activity in those cells [9]. In a subsequent experiment, it was found to reduce tremor in the harmaline rat model, possibly by reducing these oscillations [10]. Based on these results, Bushara et al. [11] conducted an initial human study. A single small dose (1 mg/kg) given to 12 patients was found to reduce tremor for 90 minutes with no intoxication. A safety study exploring doses up to 64 mg/kg found greater tremor reduction and extended time of action. Although no intoxication was observed, some patients reported mild side effects including lethargy, nausea, and asthenia [12]. Our most recent work [13] explored the pharmacokinetics of 2 formulations of 1-octanol at 64 mg/kg and demonstrated that 1-octanol was rapidly metabolized to octanoic acid. The temporal profile of octanoic acid plasma concentrations closely matched the clinical tremor reduction measured using objective spirography [14]. Based on the identification of a potentially novel category of carboxylic acid-based tremor suppression agents, our aim in this study was to test whether octanoic acid demonstrated tremor suppression properties in the rodent harmaline model of ET.

Methods

To test our hypothesis that octanoic acid suppresses harmaline-induced tremors in rodents, we used methodology of Martin et al. [15] briefly summarized below.

Protocol Male imprinting control region (ICR) mice (20-24 grams, Harlan, Indianapolis, IN) were housed in groups with free access to rodent diet and water. Experiments were approved by the Institutional Animal Care and Use Committee in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the United States National Institutes of Health. Each mouse was placed within a black plexiglass cage atop a Convuls-1 Sensing Platform model 1335-1A (Columbus Instruments, Columbus, OH) whose motion detector was connected to a Grass model P511 AC amplifier (Grass Instruments, West Warwick, RI) with 1 and 70 Hz filter settings. Digitally recorded motion power was analyzed using Spike2 software (Cambridge Electronic Design, Cambridge, UK) to perform Fourier transformation of the data into frequency spectra. Twenty minutes of pre-harmaline motion power data (baseline) were collected followed by subcutaneous injection of harmaline (Sigma-Aldrich, St. Louis, MO) with 20 mg/kg in saline 4 ml/kg, followed by another 20 minutes of motion power collection (Harmaline epoch). Data were sampled at 128 Hz. Only mice with an adequate tremor response to harmaline continued in the study. Mice were then injected intraperitoneally with octanoic acid (Sigma-Aldrich; St. Louis, MO) or 1-octanol (Spectrum Chemicals; New Brunswick, NJ) dissolved in a polyethylene glycol vehicle (PEG) (Spectrum Chemicals), or with saline or PEG alone, and motion power acquisition commenced 10 minutes later and continued for 100 minutes. The control groups (PEG or saline) used 19 to 20 mice each, whereas each dose of 1-octanol or octanoic acid used 9 to 11 animals each. To assess for potential impairment of motor functioning we used the horizontal wire [16] test in mice that did not receive harmaline. Following specific doses of 1-octanol or octanoic acid, each mouse was positioned so that the forepaws touched a 25-cm long, 2-mm diameter horizontal wire, inducing the mouse to grasp the wire. A mouse passed the test if it brought up at least one hind paw to touch the wire and did not fall off within 10 seconds of test initiation. Mice were tested at 10-minute intervals for 2 hours after drug administration.

Statistical Analysis

The index of tremor was motion power percentage (MPP), the tremor bandwidth divided by overall motion power (10-16 Hz power)/(0-34 Hz power)×100 [15]. During baseline this measure is approximately 30 to 35%, representing non-tremor movement within 10 to 16 Hz; after harmaline this value attains 60 to 80% due to the addition of tremor motion power.

An analysis of variance model was applied to MPP values for each drug followed by post-hoc *t* tests under the model using the Tukey–Fisher significance criterion. This analysis was performed with JMP (SAS, Cary, NC). For descriptive purposes, we calculated "tremor remaining" after drug or control vehicle injection using the formula (E-B)/(H-B), where E refers to MPP during the 10 to 70 minutes period post treatment, H is the pre-treatment harmaline epoch MPP, and B is the pre-harmaline epoch MPP. This calculation assumes that the nontremor MPP represented by B is constant during the experiment.

Results

We found that whereas the maximum dose of 1-octanol at which 3 of 3 mice pass the horizontal wire test was 1000 mg/kg, and it was 1500 mg/kg for octanoic acid. Harmaline tremor was relatively stable for the first hour of data acquisition after treatments. Thus, we present the MPP data based on this time period after postharmaline treatments across doses (Fig. 1). The PEG and saline control groups had very similar average MPP values at 60 to 64%, thus they are combined to provide the 0 mg/kg data points in Fig. 1 (n=39 and 38 for 1-octanol and octanoic acid, respectively). For reference, Fig. 1 displays the pre-harmaline baseline nontremor MPP and pre-treatment harmaline MPP pooled from treatment groups (n=81 and 77 for 1-octanol and octanoic acid, respectively). The increment in motion power between baseline and harmaline represents the harmaline-induced contribution of tremor. Figure 1 indicates that with increasing dosages of 1-octanol, lower MPP values occurred, indicating tremor suppresson. Compared to 0 mg/kg, statistically significant tremor suppression by 1-octanol occurred at doses as low as 30 mg/kg. The calculated average tremor remaining in the 0 m/kg group during the hour after harmaline was 77.2%. In other words, during the hour after vehicle control injections, mice showed 22.8% less tremor compared to the pre-treatment harmaline epoch. Among mice administered 1-octanol, calculated remaining tremor during the post-treatment hour fell to: 54.1% (30 mg/kg group), 37.9% (100 mg/kg), and 32.3% (530 mg/kg). The 100 mg/kg dose reduced tremor by approximately half compared to the 0 mg/kg group.

In response to octanoic acid, tremor suppression also occurred and was more robust at higher doses, but did not attain statistical significance until 300 mg/kg compared to 0 mg/kg. Average MPP values at 30 and 100 mg/kg were not statistically significantly different from those of 1-octanol at the same dose, but were less effective than 1-octanol at 300 mg/kg (p=0.040, Student's t test). The calculated average tremor remaining at 0 mg/kg was 77.8%, at 100 mg/kg it was 65.3%, and at 900 mg/kg it was 32.9%.

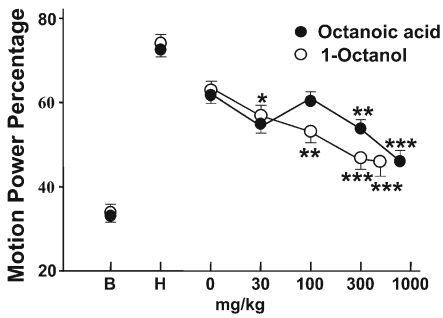

Fig. 1 Mean motion power percentage of harmaline-injected mice treated with octanoic acid or 1-octanol at 10-70 minutes following treatment. For comparison, pre-harmaline baseline (B) and pre-treatment harmaline (H) epochs are shown for each drug, averaged across all treatment groups. The motion power percentage refers to digitized motion power within the tremor bandwidth (10-16 Hz) divided by overall motion power at 0-34 Hz. Means and SEMs are shown. Statistical comparisons are with 0 mg/kg and Student's t-test. *p<0.05, **p=0.01, ***p<0.001

Discussion

These results provide further evidence in support of our earlier findings suggesting that octanoic acid has tremor suppression properties [13]. We found that with 1-octanol, octanoic acid suppresses harmaline tremor, with more of an effect at higher doses. This finding supports the notion that octanoic acid has potential for anti-tremor efficacy in ET. Although octanoic acid appeared in these experiments to be less prone to produce motor impairment and less potent than 1-octanol in our mice, this cannot be extrapolated to predict less potency for octanoic acid than for 1-octanol in humans with ET, as there are considerable differences in the kinetics of metabolism and tissue distribution between humans and mice. Another possibility is that 1-octanol acts as a pro-drug for octanoic acid, but appears to be more effective because it has less first-pass metabolism than octanoic acid. Much more needs to be learned about the kinetics and mechanisms by which 1-octanol and octanoic acid suppress tremor. Further studies are warranted to test the safety and efficacy of octanoic acid in humans, whereas exploration continues for other potential carboxylic acid-based tremor suppressants.

Disclosures

Dr. Nahab has received research support from the National Institute of Neurological Disorders and Stroke (NINDS), the International Essential Tremor Foundation, the National Parkinson Foundation, honoraria from GE Healthcare and Allergan, and he is an inventor for patent applications of 1-octanol and octanoic acid held by NINDS and the National Insitutes of Health (NIH). Mr. Brown has received financial support from the NIH and the National Hispanic Science Network. Dr. Quesada has received research support from Veterans Affairs, the International Essential Tremor Foundation, and the American Parkinson's Disease Association. Dr. Handforth has received support from Veterans Affairs, the International Essential Tremor Foundation, Medtronic, Sonexa, Forest Labs, Ortho-McNeil, and Cyberonics. Dr. Haubenberger received research grants from the Austrian Science Fund and the NINDS Intramural Research Program. Dr. Hallett serves as Chair of the Medical Advisory Board

for the Neurotoxin Institute and receives honoraria and funding for travel from the Neurotoxin Institute. Hallett may accrue revenue on U.S. Patent (#6,780,413 B2 issued on August 24, 2004): Immunotoxin (MAB-Ricin) for the treatment of focal movement disorders, and U.S. Patent (#7,407,478 issued on August 5, 2008): Coil for Magnetic Stimulation and methods for using the same (H-coil); in relation to the latter, Hallett has received license fee payments from the NIH (from Brainsway) for licensing of this patent. He receives publishing royalties from Blackwell Publisher, Cambridge University Press, Springer Verlag, Taylor & Francis Group, Oxford University Press, John Wiley & Sons, Massachusetts Medical Society, Wolters Kluwer, and Elsevier. He has received honoraria for lecturing from Columbia University and the Parkinson and Aging Research Foundation. Dr. Hallett's research at the NIH is largely supported by the NIH Intramural Program. Supplemental research funds came from the US Army via the Henry Jackson Foundation, Ariston Pharmaceutical Company via a Cooperative Research and Development Agreement (CRADA) with NIH, and the Kinetics Foundation and BCN Peptides, S.A., via Clinical Trials Agreements (CTA) with the NIH.

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Required Author Forms Disclosure forms provided by the authors are available with the online version of this article.

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