Journal of Pharmacological Sciences xxx (2018) 1–8

Contents lists available at ScienceDirect

Journal of Pharmacological Sciences

journal homepage: www.elsevier.com/locate/jphs

Full Paper

Pharmacological characterization of nicotine-induced tremor: Responses to anti-tremor and anti-epileptic agents

Naofumi Kunisawa, Saki Shimizu, Masaki Kato, Higor A. Iha, Chihiro Iwai, Mai Hashimura, Mizuki Ogawa, Shohei Kawaji, Kazuma Kawakita, Keisuke Abe, Yukihiro Ohno*

Department of Pharmacology, Osaka University of Pharmaceutical Sciences, 4-20-1 Nasahara, Takatsuki, Osaka 569-1094, Japan

ARTICLE INFO

Article history:
Received 3 April 2018
Received in revised form
18 May 2018
Accepted 23 May 2018
Available online xxx

Keywords:
Nicotine
Tremor
Anti-tremor drugs
Anti-epileptic drugs
Essential tremor

ABSTRACT

We previously showed that nicotine evoked kinetic tremor by activating the inferior olive, which is implicated in the pathogenesis of essential tremor, via α7 nicotinic acetylcholine receptors. Here, we evaluated the effects of various anti-tremor and anti-epileptic agents on nicotine-induced tremor in mice to clarify the pharmacological characteristics of nicotine tremor. Drugs effective for essential tremor, propranolol, diazepam and phenobarbital, all significantly inhibited kinetic tremor induced by an intraperitoneal (i.p.) injection of nicotine (1 mg/kg). In contrast, none of the medications for Parkinson's disease, L-DOPA, bromocriptine or trihexyphenidyl, affected the nicotine tremor. Among the anti-epileptic agents examined, valproate, carbamazepine and ethosuximide, significantly inhibited nicotine-induced tremor. In addition, a selective T-type Ca^{2+} channel blocker, TTA-A2, also suppressed the nicotine tremor. However, neither gabapentin, topiramate, zonisamide nor levetiracetam significantly affected nicotine-induced tremor. The present results show that nicotine-induced tremor resembles essential tremor not only on the neural basis, but also in terms of the pharmacological responses to anti-tremor agents, implying that nicotine-induced tremor can serve as a model for essential tremor. In addition, it is suggested that anti-epileptic agents, which have stimulant actions on the GABAergic system or blocking actions on voltage-gated Na^+ channels and T-type Ca^{2+} channels, can alleviate essential tremor.

1. Introduction

Tremor is one of the most common movement disorders in the elderly population and is often manifested as two major disorders, essential tremor and Parkinson's disease. Essential tremor and Parkinsonian tremor are fundamentally different from each other in terms of their symptoms, etiology and treatments.[1–4] Patients with essential tremor primarily exhibit kinetic tremor, while tremor in Parkinson's disease mainly appears at rest (resting tremor). In addition, causal sites or neural pathways in the brain are considered to be different between essential tremor and parkinsonian tremor, the former being the inferior olive (IO) and the latter the basal ganglia (i.e., nigro-striatal dopaminergic pathway).[5–9] Although there are many medications for Parkinson's disease (e.g., L-DOPA, dopamine D_2 receptor stimulants and anti-muscarinic agents), specific and satisfactory drugs for essential tremor are not yet available and a limited number of off-label medicines (e.g., propranolol and primidone) are clinically used to treat essential tremor.

Nicotine has a variety of pharmacological actions such as antidepressant action, cognitive enhancement, positive reinforcement and motor excitement.[10–20] We previously demonstrated that nicotine even at relatively low doses (0.5–1 mg/kg) evoked kinetic tremor in rodents via stimulating α7 nicotinic acetylcholine (nACh) receptors. In addition, brain expression analysis of Fos protein, a biological marker of neural excitation, and subsequent electrical lesion studies revealed that the IO is a specific causal site for the induction of nicotine tremor.[18,19] Since the IO is also involved in the induction of essential tremor, it seems likely that the olivocerebellar system is a common tremorgenic pathway involved both in essential tremor and nicotine-induced tremor. Despite the

* Corresponding author.
E-mail address: yohno@gly.oups.ac.jp (Y. Ohno).
Peer review under responsibility of Japanese Pharmacological Society.

https://doi.org/10.1016/j.jphs.2018.05.007

similarity in the neural basis underlying tremor generation, pharmacological characteristics of nicotine-induced tremor, especially the responses to the drugs for tremor and other motor disorders, remain to be clarified.

In the present study, we evaluated the effects of various anti-tremor agents on nicotine-induced tremor in mice to verify the pharmacological similarity between nicotine tremor and essential tremor. In addition, since several anti-epileptic agents are suggested to be effective to treat human essential tremor,[21,22] we further estimated the actions of anti-epileptic agents against nicotine-induced tremor.

2. Materials and methods

2.1. Animals

Male ddY mice (Japan SLC, Shizuoka, Japan) at 6–8 weeks of age were used. Animals were housed in air-conditioned rooms (24 ± 2 °C and 50 ± 10% relative humidity) under a 12-h light/dark cycle (light on: 8:00 a.m.) and given food and water *ad libitum*. The animal care and housing methods complied with the Guide for the Care and Use of Laboratory Animals of the Ministry of Education, Science, Sports and Culture of Japan. The experimental protocols were approved by the Experimental Animal Research Committee at Osaka University of Pharmaceutical Sciences.

2.2. Evaluation of nicotine-induced tremor

A tremorgenic dose of nicotine was set at 1 mg/kg (intraperitoneal injection (i.p.)) based on our previous dose–response results for nicotine tremor.[18] Each animal was treated with nicotine (1 mg/kg, i.p.) and individually placed in an observation chamber (25 × 42 × 20 cm). The intensity and duration of nicotine-induced tremor were measured in a time-sampling manner over 1–2, 3–4, 5–6, 7–8 and 9–10 min (each for a 1 min measurement) after nicotine injection. The tremor intensity was scored using a 4-point ranked scale, 0: none, 1: weak (mild tremor in limited regions including the forelimbs, neck, tail and head, usually with Straub tail), 2: moderate (apparent tremor in extended regions including the upper body trunk and head), 3: marked (intensive tremor over the whole body).[18,19] The total score of tremor intensity and total tremor duration were calculated as the sum of the tremor score and the tremor duration at each time point during the 10-min observation period, respectively.

2.3. Evaluation of anti-tremor agents

The following agents were evaluated as anti-tremor drugs for essential tremor; propranolol (3, 30, 60 mg/kg, i.p.), diazepam (1, 3 mg/kg, i.p.) and phenobarbital (40 mg/kg, i.p.), and for anti-parkinsonian tremor; L-DOPA (100, 300 mg/kg, i.p.), bromocriptine (10, 30 mg/kg, i.p.) and trihexyphenidyl (3, 10 mg/kg, i.p.). The test doses of each drug were set according to previous papers examining their anti-tremor or anti-parkinsonian actions.[23–30] These agents were administered to the mice 15 min before the nicotine (1 mg/kg, i.p.) injection.

2.4. Evaluation of anti-epileptic agents

The following agents were evaluated as anti-epileptic drugs; valproate (100, 300 mg/kg, i.p.), carbamazepine (10, 30 mg/kg, i.p.), ethosuximide (30, 100 mg/kg, i.p.), gabapentin (10, 30 mg/kg, i.p.), topiramate (30, 100 mg/kg, i.p.), zonisamide (10, 30 mg/kg, i.p.) and levetiracetam (100, 300 mg/kg, i.p.). The test doses were set according to previous studies examining their anti-epileptic actions.[31–36] A selective T-type Ca^{2+} channel blocker TTA-A2 (2-(4-Cyclopropylphenyl)-N-{(1R)-1-[5-(2,2,2-trifluoroethoxy)pypyridin-2-yl]ethyl}acetamide) was also tested to confirm the involvement of T-type Ca^{2+} channels in modulating the nicotine tremor. These agents were administered to the mice 15 min before the nicotine (1 mg/kg, i.p.) injection.

2.5. Drugs

(−)-Nicotine, (±)-propranolol hydrochloride, L-3,4-dihydroxyphenylalanine methyl ester hydrochloride (L-DOPA), 2-bromo-α-ergocryptine methanesulfonate salt (bromocriptine), trihexyphenidyl hydrochloride, valproic acid sodium salt and ethosuximide were purchased from Sigma–Aldrich (St. Louis, MO). Phenobarbital (Phenobal) was purchased from Daiichi Sankyo Co. Ltd (Tokyo, Japan). Diazepam (Cercine) was purchased from Takeda Pharmaceutical Co. Ltd (Osaka, Japan). Carbamazepine, gabapentin, topiramate, levetiracetam and zonisamide were purchased from Tokyo Kasei Kogyo Co. (Tokyo, Japan). TTA-A2 was purchased from Alomone Labs (Jerusalem, Israel). Propranolol was first dissolved in 1% lactate solution and diluted with saline (final concentration: 0.3%). Phenobarbital, diazepam and carbamazepine were first dissolved in polyethylene glycol (PEG) and diluted with distilled water (final concentration: 10%). Bromocriptine, topiramate, zonisamide and TTA-A2 were first dissolved in dimethyl sulfoxide (DMSO) and diluted with distilled water (final concentration: 20%). Other agents were dissolved in physiological saline. Control animals were treated with respective vehicle alone.

2.6. Statistical analysis

Data are expressed as the mean ± S.E.M. Statistical significance of differences among multiple groups was determined by the Kruskal–Wallis test followed by the Steel-Dwass post-hoc comparison test. The time-course data were analyzed by two-way repeated measures ANOVA followed by the Tukey post-hoc comparison test. Comparisons between only two groups were performed by Mann–Whitney's U-test. A P value of less than 0.05 was considered statistically significant.

3. Results

3.1. Effects of anti-essential tremor drugs on nicotine-induced tremor

We first examined the effects of drugs for essential tremor, propranolol (a β receptor antagonist), diazepam (a benzodiazepine-binding site agonist of $GABA_A$ receptor) and phenobarbital (a barbiturate-binding site agonist of $GABA_A$ receptor), on nicotine-induced tremor. As reported previously, nicotine at 1 mg/kg (i.p.) evoked transient and kinetic tremor in all mice tested, which usually subsided within 15 min after the nicotine injection. Treatment of mice either with propranolol (60 mg/kg, i.p.), diazepam (3 mg/kg, i.p.) or phenobarbital (40 mg/kg, i.p.) suppressed the induction of nicotine tremor (Fig. 1). The total score and duration of nicotine-induced tremor during the 10 min-observation were significantly reduced by propranolol at 3 mg/kg (i.p.) (total score: $U(11) = 6$, $P = 0.0277$ and total duration: $U(11) = 6.5$, $P = 0.0381$) and at 30 and 60 mg/kg (i.p.) (total score: $\chi^2 = 11.9959$, df = 2, $P = 0.0025$ and total duration: $\chi^2 = 12.6827$, df = 2, $P = 0.0018$) (Fig. 2A). Diazepam (1 and 3 mg/kg, i.p.) (total score: $\chi^2 = 12.1006$, df = 2, $P = 0.0024$ and total duration: $\chi^2 = 12.4150$, df = 2, $P = 0.0020$) and phenobarbital (40 mg/kg, i.p.) (total score: $U(14) = 9$, $P = 0.0123$ and total duration: $U(14) = 10$, $P = 0.0208$) also significantly inhibited nicotine-induced tremor (Fig. 2B and C).

Please cite this article in press as: Kunisawa N, et al., Pharmacological characterization of nicotine-induced tremor: Responses to anti-tremor and anti-epileptic agents, Journal of Pharmacological Sciences (2018), https://doi.org/10.1016/j.jphs.2018.05.007