Pharmacological Reports 67 (2015) 435–441

Contents lists available at ScienceDirect

Pharmacological Reports

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

Original research article

Apomorphine enhances harmaline-induced tremor in rats

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ARTICLE INFO

Article history:
Received 4 August 2014
Received in revised form 8 November 2014
Accepted 13 November 2014
Available online 27 November 2014

Keywords:
Tremor
Harmaline
Apomorphine
Propranolol
Rat

ABSTRACT

Background: Harmaline-induced tremor is a well-known model of essential tremor in humans. The aim of the present study was to examine the influence of apomorphine, a non-selective dopamine receptor agonist, on the tremor induced by harmaline in rats. Propranolol (a first-line drug in essential tremor) was used as a reference compound.

Methods: Tremor, locomotor activity and focused stereotypy were measured objectively using force plate actimeters. Tremor was analyzed using a Fourier transform to generate power spectra for rhythmic behavior.

Results: The tremor induced by harmaline administered at a dose of 15 mg/kg *ip* was associated with an increase in power in the 9–15 Hz band (AP2) and in the tremor index, calculated as a difference between AP2 and power in the 0–8 Hz band (AP1). Propranolol injected at a dose of 20 mg/kg *ip* reversed both of these effects of harmaline. Apomorphine administered at the doses of 0.5 and 1 mg/kg *sc* further enhanced AP2 and at the lower dose also the tremor index elevated by harmaline. This increase in AP2 was stronger than enhancement of locomotor activity induced by apomorphine in the harmaline-treated animals.

Conclusions: The present study suggests that the dopamine agonist apomorphine enhances the tremor induced by harmaline, and this effect is at least partly independent of hyperactivity.

Introduction

Essential tremor (ET) is a frequent neurological disorder. ET etiology is often genetic but environmental toxins have been proposed as risk factors. Neuronal mechanisms underlying ET are poorly understood and at present the medication for treatment of ET is unsatisfactory [1].

Harmaline, a β-carboline derivative is a commonly used compound to model ET in animals and screen potential tremorolytic drugs [2,3]. In rats, a harmaline-induced kinetic/postural tremor has an oscillation frequency of 10–12 Hz [4]. The primary cause of this disorder [4] is synchronous activation of the olivocerebellar glutamatergic climbing fibers [5], which leads to glutamate release in the cerebellum [6,7], and to an enhancement of complex spike activity of Purkinje cells of the cerebellar cortex [8].

Several studies suggest that the excitability of cerebellar neurons may be influenced by dopaminergic transmission. For example, the cerebellar cortex receives sparse dopaminergic input from the mesencephalon [9,10]. Moreover, Purkinje cells express tyrosine hydroxylase [11], and release dopamine, which then excites them *via* dopamine D3 autoreceptors [12]. A dopamine D3 receptor agonist [13] or chronic blockade of dopaminergic receptors [14] can increase expression of *c-fos/c-Fos* in the cerebellum. Furthermore, lesions of the dopaminergic nigrostriatal pathway induce metabolic activation of the cerebellum [15], and increase firing of Purkinje cells [16], whereas stimulation of the substantia nigra elevates cerebellar c-Fos immunoreactivity [14].

The role of dopaminergic neurotransmission in harmaline-induced tremor and ET is unclear. A moderate lesion of dopaminergic mesencephalic neurons has been found to modulate tremor in rats [7,17]. Such a lesion increased the tremor induced by a low dose of harmaline [17] but inhibited the tremor induced by a very high dose [7]. Activation of the dopaminergic neurons that survive the aforementioned lesion [17] suggested that the modulatory effects of the lesion on tremor resulted from a counterbalance between losses of dopaminergic transmission and activation of compensatory mechanisms. Furthermore, Paterson and co-workers [3] reported an inhibition of harmaline-induced tremor after systemic injections of dopamine agonists, apomorphine, quinpirole, and a dopamine reuptake inhibitor. Previously, Costall et al. [18] reported similar effects of dopamine agonists

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http://dx.doi.org/10.1016/j.pharep.2014.11.008
1734-1140/

with respect to the tremor induced by harmine (a harmaline metabolite). However, these latter results cast doubt on the usefulness of β-carboline models to screen drugs for ET, due to the lack of any data clearly showing the clinical effectiveness of dopamine agonists [1]. In contrast, the therapeutic effect of atypical neuroleptics in ET is well established [1].

Tremor is often difficult to quantify and distinguish from other motor abnormalities in animals, which may be problematic for research. For example, we recently found, using fully automatic force plate actimeters, that in addition to tremor, harmaline induces delayed general hyperactivity (bouts of low mobility, increased basic activity) [19], which might interfere with the scoring of tremor intensity. Therefore, in the present study we used a similar approach to re-examine the influence of apomorphine on the harmaline-induced tremor in rats while simultaneously measuring locomotor activity and stereotypy. Propranolol (the first-line treatment of ET [1]) was used as a reference compound.

Materials and methods

Animals

The experiments were carried out in compliance with the Animal Experiments Bill of January 21, 2005 (published in Journal of Laws no. 33/2005 item 289, Poland), and according to the EC Directive 86/609/EEC on the protection of animals used for scientific purposes. Additionally, the experiments were approved by the Local Ethics Committee. All efforts were made to minimize the number and suffering of animals used. Male Wistar rats weighing 260–390 g prior to the experiments were kept under a 12/12 h light/dark cycle (the light on from 7 am to 7 pm) with free access to food and water. All experiments were carried out during the light period.

Drugs

Harmaline hydrochloride dihydrate (Sigma) was administered at a dose of 15 mg/kg *ip*. R-(−)apomorphine hydrochloride hemihydrate (Sigma, 0.5 and 1 mg/kg *sc*) or (±)propranolol hydrochloride (Sigma, 20 mg/kg *ip*) was injected simultaneously with harmaline. The above drugs were dissolved in redistilled water. Control animals received physiological saline twice instead of harmaline, propranolol or apomorphine. A dose of harmaline (15 mg/kg) was used following our recent paper [19], which showed that this dose induced marked tremor in rats, as measured by the force plate actimeters. The doses of apomorphine (0.5 and 1 mg/kg) and propranolol (20 mg/kg) were chosen according to previous studies that characterized their effectiveness in harmaline-induced tremor in rodents [2,3]. These doses of apomorphine evoked stereotypy (repetitive head movements, sniffing, licking, gnawing and biting) and locomotor stimulation in rodents [20–22], whereas that of propranolol decreased exploratory activity of rats [23].

Force plate actimeters

Professor Stephen C. Fowler and Troy Zarcone of the University of Kansas (Lawrence, KS, USA) developed the Force Plate Actimeters (BASi). The concept and applications of this instrument for measurement of tremor, locomotor activity and stereotypy in rodents were described in the USA and pending international patents (USA Patent no. 6,601,010), as well as in published research [24–29].

Immediately after drug injections, rats were placed in force plate actimeters and behavioral parameters were measured during 60 min. An animal was placed on a 44 cm × 44 cm plate covered by a Plexiglas enclosure (33 cm height) and put into a ventilated sound-attenuating chamber. The force plate actimeters tracked the animals' movements across a plate. Four force transducers below the corners of the plate recorded the animal's position on a Cartesian plane and measured the force exerted on the plate at each time point. Data were collected and stored during time units of 40.96 s ("frames") with the sampling frequency 50 points per second, and accompanying software analysis allowed the user to apply the data for analysis of specific behaviors of interest.

Tremor was analyzed using Fast Fourier Transform (FFT) on each frame of the experiment. Then, the resulting power spectra were log base 10 transformed and averaged over six consecutive 15-frame series [six time periods of *ca.* 10 min each (614.4 s)] to give the following parameters: AP1 – averaged power in frequency band I (0–8 Hz); AP2 – averaged power in frequency band II (9–15 Hz); the tremor index – the difference between the averaged power in the band II and the averaged power in the band I (Fig. 1).

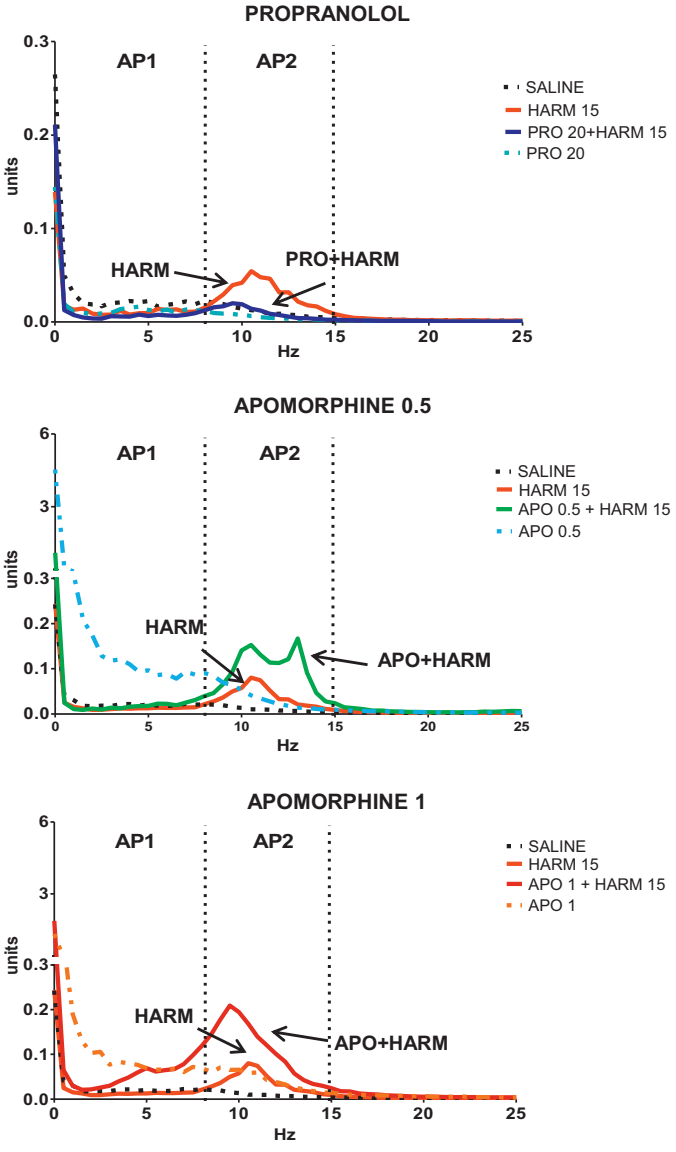

Fig. 1. Propranolol and apomorphine influence harmaline-induced tremor. The power spectrum for the harmaline-induced tremor within a range of 0–25 Hz averaged for the whole period of the experiment (60 min) and for all animals. AP1 – power in the 0–8 Hz band; AP2 – power in the 9–15 Hz band; SALINE – controls; HARM 15 – harmaline 15 mg/kg; APO 0.5, 1 – apomorphine 0.5 and 1 mg/kg; PRO 20 – propranolol 20 mg/kg.