EFFECTS OF CHRONIC MAFEDINE EXPOSURE IN ZEBRAFISH

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Abstract

Pharmacological agents acting at alpha-2 adrenergic receptors (Table 1) are widely used in cardiology and neuroscience research. Mounting evidence suggests their potential utility in clinical and experimental psychopharmacology, necessitating novel models and novel model organisms for their screening.

In this study the investigated the effects of chronic 6-oxo-1-phenyl-2- (phenylamino)-1,6-dihydropyrimidine-4-sodium olate (mafedine), alpha-2 adrenergic receptor agonist, exposure on zebrafish behaviour in the Novel tank test. The studied compound was administrated at the doses of 1 mg/L, 5 mg/L and 10 mg/L once daily during the 7 days.

Madedine at 1 mg/L had a **psychostimulating** action, whereas higher doses caused **anxiogenesis** in zebrafish.

Introduction

The novel compound, mafedine, is a pyrimidine derivative 6-oxo-1-phenyl-2- (phenylamino)-1,6-dihydropyrimidine-4-olate sodium (Fig. 1) synthesized in the Organic Chemistry Department of St. Petersburg State Chemical Pharmaceutical Academy (St. Petersburg, Russia). The drug is a white crystalline powder with pink tinge, highly soluble in water.

In a series of studies of its antihypertensive effects, mafedine activity was mediated via central alpha-2 adrenergic receptors. Therefore, similar to clonidine and other alpha-2 agonists, mafedine may be effective in certain neurological and mental conditions.

Capitalizing on the zebrafish model as a sensitive in vivo system for CNS drug discovery, here we examine the effects of chronic administration of mafedine on adult zebrafish.

Materials and methods

The study was performed in adult zebrafish. Behavioral testing was performed using the Novel tank test.

Mafedine was administrated at the doses of 1 mg/L, 5 mg/L and 10 mg/L once daily during the 7 days. Animals were randomly divided on 4 experimental groups, 15 fish in each of them: control, mafedine 1, 5 and 10 mg/L. Trials were recorded for 5 min by web-camera for further analyses.

For each animal, the distance travelled (cm), the mean and maximum velocity (cm/s) and turn angle (deg) were estimated. Also the frequency and duration (s) of low mobility (the complete area detected as animal is changing with <20% threshold), high mobility (the complete area detected as animal is changing with >60% threshold) and not moving state (no changes of location, threshold was defined as: start velocity – 2.00 cm/s, stop velocity – 1.75 cm/s), the time spent in the top and in the bottom zones (s), the frequency of transition from bottom to top and latency time of the first bottom-top transition (s).

The **statistical significance** of differences between groups was assessed using the Kruskal-Wallis test followed by Dunn´s post-hoc test. The level of confidence was set as 95%.

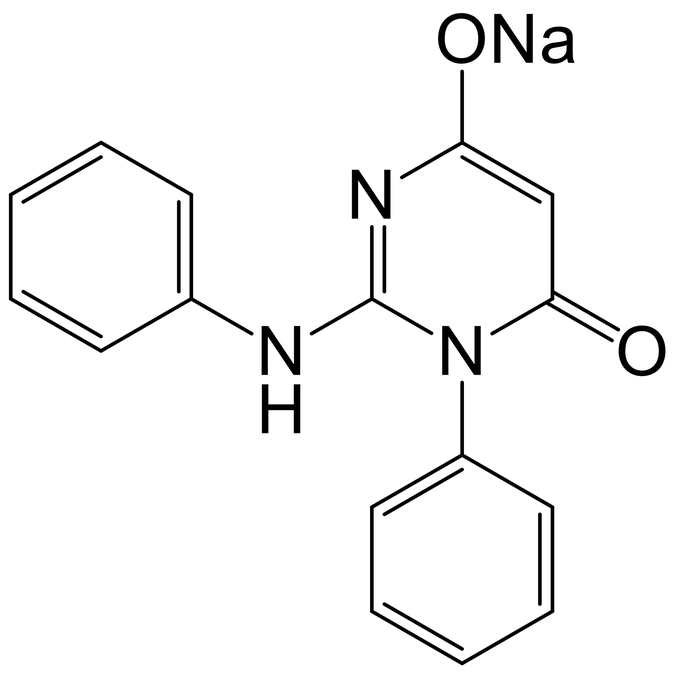
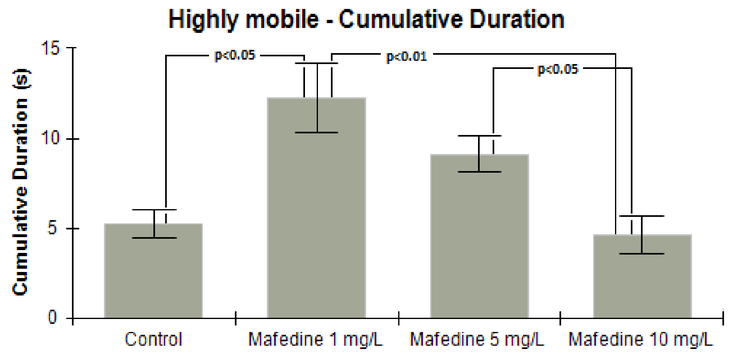
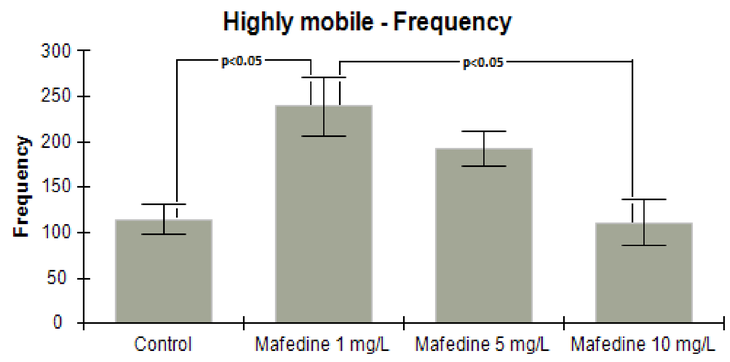
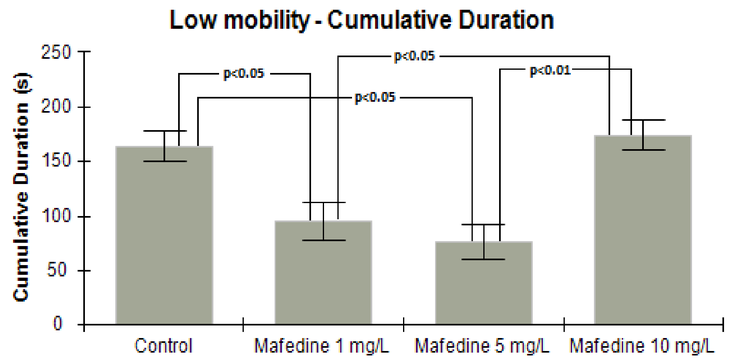


Fig 1. The structure of mafedine







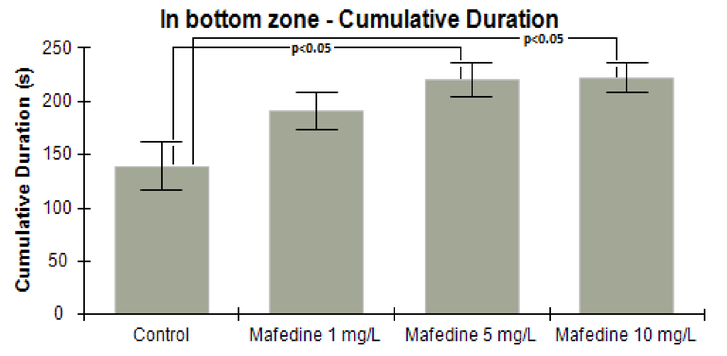


Table 1. CNS localization, known physiological functions and genetic homology of different alpha-2 adrenergic subtypes in zebrafish and rodents (NA – data not available). ***https://blast.ncbi.nlm.nih.gov/Blast.cgi***

Receptor subtypes							
2A		2B		2C		2Da/2Db	
Localization	Function	Localization	Function	Localization	Function	Localization	Function
Zebrafish							
Ventral telencephalon, preoptic, pretectal and hypothalamic areas, oculomotor nucleus, locus coeruleus, medial raphe, reticular formation, Purkinje layer of the cerebellum(Ampatzis et al. 2008)	NA	Hypothalamus (Ruuskanen et al. 2005b)	NA	Diencephalon (torus lateralis and optic tectum) (Ruuskanen et al. 2005b)	NA	Cerebellum, optic tectum (2Da) (Ruuskanen et al. 2005b)	NA
% homology with mice*							
Gene	81%		82%		85%	NA	NA
Protein	59%		52%		66%	NA	NA
% homology with humans*							
Gene	77%		78%		**	NA	NA
Protein	58%		68%		61%	NA	NA
Rodents (mice, rats)							
Medulla (MacMillan et al. 1996), locus coeruleus (Kable et al. 2000), amygdala, hypothalamus, olfactory system (Wang et al. 1996), both horns of the spinal cord (Shi et al. 1999)	Hypotension (MacMillan et al. 1996), nociception, hypothermia (Hunter et al. 1997) and sedation (Kable et al. 2000)	Thalamus, Purkinje layer of the cerebellum (Wang et al. 1996)	Regulation of vascular tone (Makaritsis et al. 1999)	Striatum (Wang et al. 1996), locus coeruleus (Osborne et al. 2002), ventral horns of the spinal cord (Shi et al. 1999)	Stress behavior (Sallinen et al. 1999), memory and spatial navigation (Björklund et al. 2000)	NA	NA
% homology with humans*							
Gene	83%		84%		87%	NA	NA
Protein	92%		83%		92%	NA	NA

Results and discussion

Chronic mafedine exposure at the dose of 1 mg/L led **to reduce of low mobility state duration** (H=18.711, df=3, p=0.045) in comparison with control fish. Also in this group the **increase of high mobility state frequency** (H=14.203, df=3, p=0.049) **and duration** (H=17.473, df=3, p=0.026) was observed. The higher doses of mafedine **decreased the frequency of high mobility** state **and prolonged the time spent in the bottom zone of the tank**, moreover these effects were dose-dependent.

Generally, as mafedine increased the frequency and cumulative duration of high mobility state and decreased the low mobility state duration it can be assumed that studied compound has a psychostimulant action. Higher doses led to a rise of time spent in the bottom zone of the tank by fish, so it is a hallmark of anxiogenic action. It is important to point out that observed effects of mafedine were dose-dependent. In total, effects of chronic mafedine administration are similar as after 20-min acute exposure of mafedine at the dose of 60 mg/L, which had been previously reported.

Contacts

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