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Research Article

In vitro effects of synthetic muscimol and an extract from *Amanita muscaria* on human recombinant MAOB enzyme

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Abstract

The effects of synthetic muscimol and an extract from *Amanita muscaria*, containing this compound on the activity of human recombinant MAOB enzyme (hMAOB) were studied. Muscimol had statistically significant inducing effect on hMAOB at concentrations 0.25–5 μM, while *A. muscaria* extract did not influence the enzyme activity at all.

Keywords

Amanita muscaria, muscimol, MAOB enzyme

Introduction

Monoamine oxidase (MAO) catalyses the oxidative deamination of monoamines (neurotransmitters, dietary amines, hormones and drugs) in the brain and peripheral tissues, regulating their levels and thus their biological effects. MAO exists in two isoforms, MAOA and MAOB. MAOA selectively deaminates serotonin, whereas MAOB selectively deaminates phenylethylamine and benzylamine. Dopamine is oxidised by both isoforms (Youdim et al. 2006). Both enzymes catalyse the oxidative deamination of substrates through a FAD-dependent mechanism that releases hydrogen peroxide, ammonia and an aldehyde product (Ramsay and Albreht 2018). MAOA is targeted for treatment of depression and anxiety, whereas MAOB – against Alzheimer's and Parkinson's diseases. Moreover, several recent studies have proved the role of MAOs (more specifically MAO A induced oxidative stress) in cardiovascular diseases (Mialet-Perez et al. 2018).

The main psychoactive component in *Amanita muscaria* (fam. Amanitaceae) (Bas 1969) is muscimol. Other psychoactive substances are ibotenic acid and muscazone. Approximately 10–20% of the dose of ibotenic acid ingested is converted to muscimol after decarboxylation. Ibotenic acid, unlike muscimol, is much more dangerous, causing ibotenate-induced seizures and lesions in specific brain regions, similar to Alzheimer's disease for which it is used in animal test models (Stebelska 2013). When ingested the mushroom leads to a state resembling alcohol intoxication (Meyer and Quenzer 2005), known as 'pantherina-muscaria' poisoning syndrome. Muscimol is a non-selective $GABA_A$ receptor agonist activating both pre- and postsynaptic receptors and partial agonist of GABAc receptors devoid of effects

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on the GABA-metabolizing enzyme, $GABA_A$ transaminase, and the $GABA_A$ uptake systems, which also enters the brain after peripheral injection (Snodgrass 1978). There are data about its role as a potent agonist at bicuculline-sensitive, strychnine-insensitive postsynaptic receptors of the mammalian central nervous system. For example, muscimol (rat, 3 mg/kg, i.p.) evokes serotonin rise and decreases catecholamine levels in the brain. The compound binds to $GABA_A$ receptors mainly in areas of the forebrain, including the *caudate nucleus* and putamen, the thalamus and the hippocampal formation leading to the opening of the receptor associated with the chloride ion channel, which in turn leads to inhibition of neuronal activity, where these receptors are located (Stebelska 2013).

The aim was to compare the effects of *Amanita muscaria* extract and synthetic muscimol on human recombinant MAOB enzyme activity.

Materials and methods

Extraction and determination of muscimol

A. muscaria caps were collected in May 2018 in Vitosha Mountain and identified by Vladimir Vazharov. A voucher specimen was deposited in his personal collection (Vazharov 2016). The material (100 g) was dried at room temperature, cut in pieces and then macerated with 70% ethanol (1:1) for 21 days. The tincture was filtered and used for the experiments. Waters HPLC system (Milford, MA, USA) equipped with binary gradient pump model 1525 EF, manual injector Rheodyne 7725i with 20 µl loop, UV–vis detector model 2489 and Breeze 2 software was used. An ODS column (Luna 250 × 4.6 mm, 5 µm, Phenomenex, USA) with a column guard (at 25 °C) and a mobile phase consisted of a 5 mM formate buffer (pH 7.0, A) and acetonitrile (B) with a flow rate of 1 ml/min were used. The gradient program was: initial 10% B; from 5 to 20 min 10%→100% B, linear; from 20 to 25 min maintained at 100% B; from 26 to 30 min back to 10% B, linear. Separations were monitored at 230 nm.

Muscimol CRS (Sigma Aldrich, Germany) was dissolved in 50% MeOH (2 mg/ml). Serial dilutions were made as follows: 1 mg/ml; 0.5 mg/ml; 0.1 mg/ml. An aliquot of each standard solution (10 µl) was injected three times in the HPLC. An aliquot of the tincture (10 µl), filtered through a PVDF filter (0.22 µl) was injected three times for HPLC analysis. Muscimol content was in the tincture 2.94 ± 0.03 mg/ml.

Measurement of Monoamine oxidase B activity

Monoamine oxidase activity assay of recombinant human MAOB (hMAOB) was performed using a fluorometric method by Amplex UltraRed reagent (Bautista-Aguilera et al. 2014) with small modifications (Kasabova-Angelova et al. 2020). Tyramine hydrochloride, used as the substrate, and human recombinant MAOB enzyme were obtained from Sigma Aldrich (Germany). Amplex UltraRed Kit was obtained by Invitrogen (USA).

The hMAOB was incubated for 2 h with *A. muscaria* extract (at 5 µg/ml, 1 µg/ml, 0.5 µg/ml, 0.25 µg/ml, 0.2 µg/ml, 0.1 µg/ml, 0.05 µg/ml, 0.025 µg/ml, 0.02 µg/ml, and 0.01 µg/ml muscimol) and Muscimol (at 5 µM, 1 µM, 0.5 µM, 0.25 µM, 0.2 µM, 0.1 µM, 0.05 µM, 0.025 µM, 0.02 µM, 0.01 µM) at 37 °C in the dark.

Statistical analysis

The MAOB activity was expressed as a normalized percent of the untreated control set as 100% and the results were expressed as mean values and standard deviation (± SD) (Graph Pad Prizm, v. 6). Statistical analysis was performed by one-way analysis of variance (ANOVA) with *post hoc* multiple comparisons procedure (Dunnet's test) to assess the statistical differences in case of normal distribution. Values of $P < 0.05$, $P < 0.01$ and $P < 0.001$ were considered statistically significant.

Results and discussion

Oxidative stress induced by MAO is a potential risk factor for neuronal loss in aging and age-related neurodegenerative disorders, such as Parkinson's disease (PD). Indeed, oxidative stress generated by increased MAOB activity is known to damage mitochondrial DNA (Hauptmann et al. 1996) and to reduce respiratory capacity (Kumar et al. 2003). It has been shown that an elevation in MAOB in astrocytes results in PD pathology in a mouse model (Mallajosyula et al. 2012). Inhibition of MAO in the brain increases the content of amines, resulting in improved neuronal activity and antidepressant effects (Youdim et al. 2006; Fisar 2016). In this study synthetic muscimol exerted concentration-dependent inducing effect on hMAOB activity.

It increased statistically significant the hMAOB activity at the highest concentrations: 0.25 – 5 µM, compared to the control (pure hMAOB). Five µM muscimol increased hMAOB activity about five times; 1 µM – with 169 %; 0.5 µM – with 44 % and 0.25 µM – with 27 %.

This paradox of enzyme induction could be explained with the hMAOB protein conformation change induced by high levels of positively-charging molecule such as muscimol. Single-charged and especially double charged small molecules, incl. muscimol could induce changes in the active center and/or activator-binding site of many enzymes (Kovermann et al. 2017). The lowest concentrations (0.2–0.01 µM) had no effect on hMAOB (Figure 1).

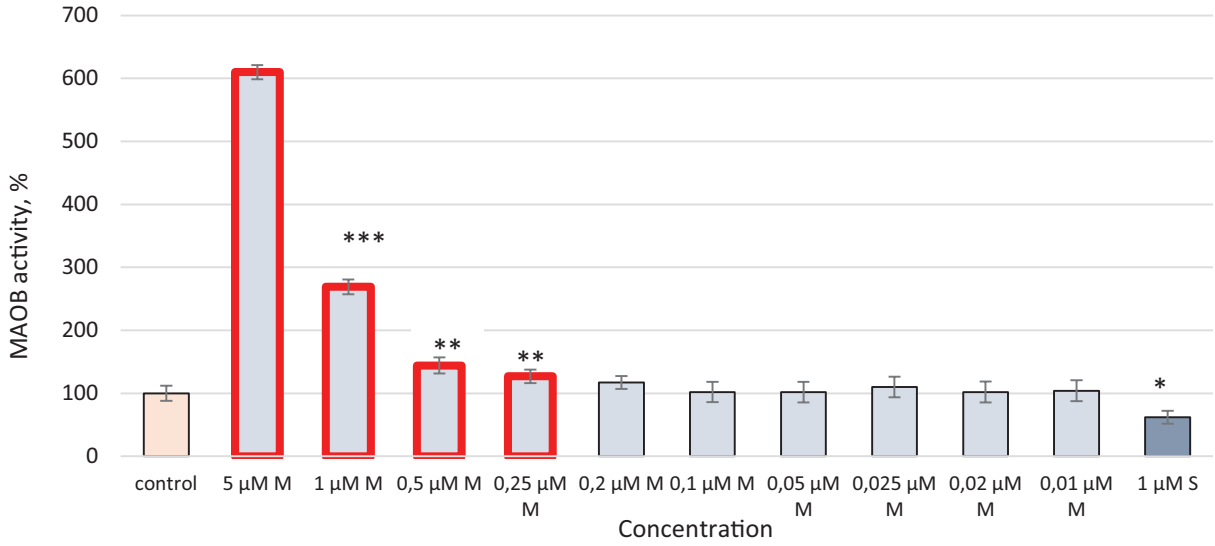

Figure 1. Effects of muscimol (M) (at different concentrations) and selegiline (S) on hMAOB activity; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ vs control (pure hMAOB).

The classical MAOB inhibitor – selegiline decreased hMAOB activity with 38%, compared to the control (pure hMAOB enzyme).

The extract from *A. muscaria* had no effect whatsoever on human recombinant MAOB enzyme (Figure 2).

Only the classical MAOB inhibitor – selegiline decreased hMAOB activity with 38 %, compared to the control (pure hMAOB enzyme).

The discovery of novel classes of selective inhibitors of this enzyme is of considerable interest. Identifying MAOB as a potential pathogenic factor in PD stimulated the development of MAOB inhibitors as disease-modifying agents; selegiline and rasagiline, which showed protective role over neuronal cells in both *in vitro* and *in vivo* models (Ebadi et al. 2006; Youdim et al. 2006; Naoi et al. 2013). MAO inhibitors have been approved as an adjunctive therapy in PD for many years, helping to preserve the diminishing dopamine and so delaying the need to start L-DOPA treatment.

Moreover, preliminary data show that *in vitro* at concentration higher than physiological, MAO deaminates GABA (Goldberg et al. 2014).

Our findings confirmed the significance of naturally-derived psychoactive alkaloids such as muscimol as leading molecules for possible treatment of neurodegenerative diseases.

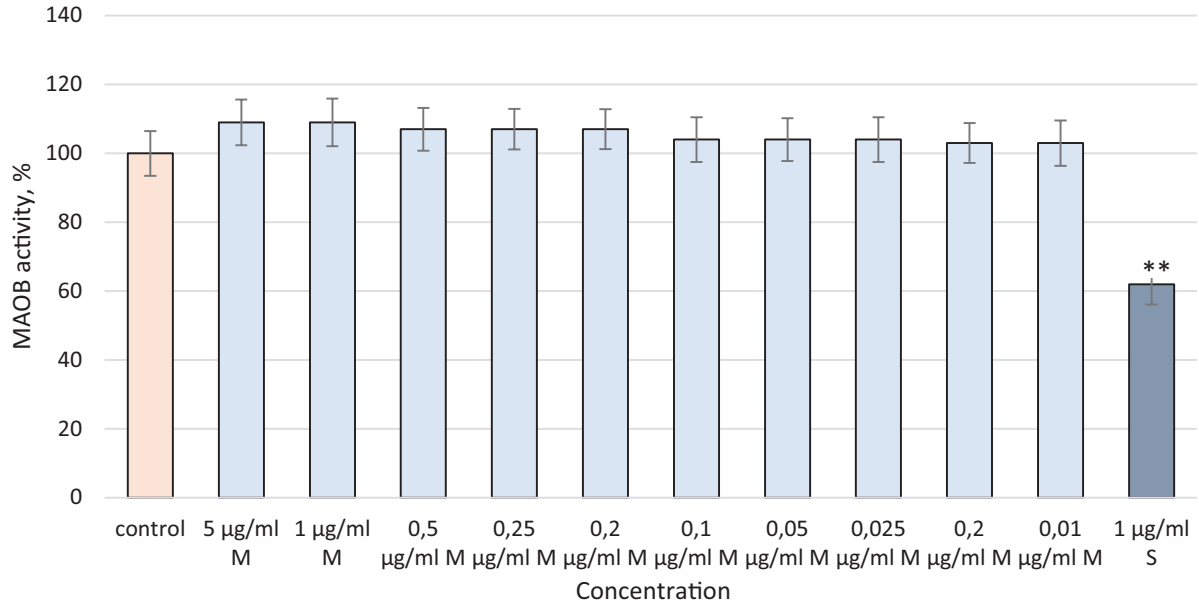

Figure 2. Effects of *Amanita muscaria* extract (at different concentrations muscimol) and Selegiline (S) on hMAOB activity. ** $P < 0.01$ *vs* control (pure hMAOB).

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