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 muscatoine. 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
on the GABA-metabolizing enzyme, GABA\textsubscript{\textalpha} transaminase, and the GABA\textsubscript{\textalpha} 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\textsubscript{\textalpha} 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).
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 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).

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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References

Bas C (1969) Morphology and subdivision of Amanita and a monograph on its section Lepidella. Persoonia 5(4): 285–579. https://pdfs.semanticscholar.org/2b12/a3b85fa3b4407d4df95e06f72a455068.pdf?_ga=2.254597707.237837792.1590850352-857800512.1575727087tp://www.persoonia.org/
Bautista-Aguilera O, Esteban G, Bolera I, Nikolic K, Agbaba D, Moraleda I, Iriepa I, Samadi A, Soriano E, Unzeta M, Contelles JM (2014) Design, synthesis, pharmacological evaluation, QSAR analysis, molecular modeling and ADMET of novel donepezil-indolyl hybrids as multipotent cholinesterase/monoamine oxidase inhibitors for the potential treatment of Alzheimer’s disease. European Journal of Medicinal Chemistry 75: 82–95. https://doi.org/10.1016/j.ejmech.2013.12.028
Ebadi M, Brown-Borg H, Ren J, Sharma S, Shavali S (2006) Therapeutic efficacy of selegiline in neurodegenerative disorders and neurological diseases. Current Drug Targets 7(11): 1513–1529. https://doi.org/10.2174/1389450110607011513
Fišar Z (2016) Drugs related to monoamine oxidase activity. Progress in Neuropsychopharmacology and Biological Psychiatry 69: 112–124. https://doi.org/10.1016/j.pnpb.2016.02.012
Goldberg J, Bell C, Pollard D (2014) Revisiting the monoamine hypothesis of depression: a new perspective. Perspectives in Medicinal Chemistry 6: 1–8. https://doi.org/10.4137/PMC.S11375
Hauptmann N, Grimsby J, Shih C, Cadenas E (1996) The metabolism of tyramine by monoamine oxidase A/B causes oxidative damage to mitochondrial DNA. Archives of Biochemistry and Biophysics 335(2): 295–304. https://doi.org/10.1006/abbi.1996.0510
Kasabova-Angelova A, Kondeva-Burdina M, Mitkov J, Georgieva M, Tzankova V, Zlatkov A (2020) Neuroprotective and MAOB inhibitory effects of a series of caffeine-8-thioglycoloc acid amides. Brazilian Journal of Pharmaceutical Sciences 56(1–9): e18255. https://doi.org/10.1590/s2175-97902019000318255
Kovermann M, Grundström C, Sauer-Eriksson E, Sauer U, Wolf-Watz M (2017) Structural basis for ligand binding to an enzyme by a conformational selection pathway. Proceedings of the National Academy of Sciences of the United States of America 114(24): 6298–6303. https://doi.org/10.1073/pnas.1700919114
Kumar J, Nicholls D, Andersen J (2003) Oxidative alpha-ketoglutarate dehydrogenase inhibition via subtle elevations in monoamine oxidase B levels results in loss of spare respiratory capacity: implications for Parkinson’s disease. Journal of Biological Chemistry 278(9): 46432–46439. https://doi.org/10.1074/jbc.M306378200
Mallajosyula J, Kaur D, Chinta J, Rajagopalan S, Rane A, Nicholls G, Di Monte A, Macarthur H, Andersen J (2012) MAO-B elevation in mouse brain astrocytes results in Parkinson’s pathology. PLoS ONE 7(8): 10.1371. https://doi.org/10.1371/annotation/3c37bef4-bb5e-4f1e-8551-01ac006d90
Meyer J, Quenzer L (2005) Psychopharmacology: drugs, the brain, and behaviour Sunderland, Massachusetts: Sinauer Associates Inc. https://books.google.bg/books/about/Psychopharmacology.html?hl=en&gbpv=1&dq=Meyer+Quenzer&printsec=frontcover
Mialet-Perez J, Manzelia N, Santin Y, Maggiorani D, Martini H, Douin-Echinard V, Passos F (2018) Monoamine oxidase-A is a novel driver of stress-induced premature senescence through inhibition of parkin-mediated mitophagy. Aging Cell 17(5): e12811. https://doi.org/10.1111/acel.12811
Naoi M, Maruyama W, Inaba-Hasegawa K (2013) Revelation in neuroprotective functions of rasagiline and selegiline: the induction of distinct genes by different mechanisms. Expert Review of Neurotherapeutics 13(6): 671–684. https://doi.org/10.1586/ern.13.60
Ramsay R, Albretch A (2018) Kinetics, mechanism, and inhibition of monoamine oxidase. Journal of Neural Transmission 125(11):1659–1683. https://doi.org/10.1007/s00702-018-1861-9
Snodgrass S (1978) Use of 3H-muscimol for GABA receptor studies. Nature 273(5661): 392–394. https://doi.org/10.1038/273392a0
Stebelska K (2013) Fungal hallucinogens psilocin, ibotenic acid, and muscimol. Therapeutic Drug Monitoring 35(4): 420–442. https://doi.org/10.1097/FTD.0b013e318238741a5
Vazharov V (2016) Medicinal Fungi in Bulgaria. Biana, Sofia.
Youdim M, Bakhle Y (2006) Monoamine oxidase: isozymes and inhibitors in Parkinson’s disease and depressive illness. British Journal of Pharmacology 147(1): 287–296. https://doi.org/10.1038/sj.bjp.0706464
Youdim M, Edmondson D, Tipton K (2006) The therapeutic potential of monoamine oxidase inhibitors. Nature Reviews Neuroscience 7(4): 295–309. https://doi.org/10.1038/nrn1883