The impact of methylphenidate and its enantiomers on the dopaminergic synthesis and metabolism in-vitro

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Introduction

Attention-deficit/hyperactivity disorder (ADHD) is one of the most frequent psychiatric disorder in children and adolescents. ADHD is characterized by developmentally inappropriate levels of inattention, hyperactivity and/or impulsivity. Stimulants are part of the standard-of-care treatment for ADHD. Racemate of d/l-threo-methylphenidate (MPh; Ritalin) is an effective first-line treatment for the symptoms associated with attention-deficit/hyperactivity disorder (ADHD). Although MPH has long been administered as a racemic mixture of the two enantiomers, converging lines of evidence drawn from investigations using in vitro systems indicate that it is predominantly, d-threo MPH which mediates the pharmacological/therapeutic actions of MPH. In the present study, we investigated the MPH influence on the tyrosine hydroxylase (TH), monoamine oxidase B (MAO-B), and aldehyde dehydrogenases (ALDH) enzyme activity in vitro, as all enzymes are important for dopamine efficacy.

Methods

Protein solution from PC12 cells

Pellets of cultured rat pheochromocytoma cells (PC12) was dissolved in Complete-Lysis-M buffer (Roche, Grenzach, Germany) and homogenized via tissue lyser (Qiagen, Germany) with a frequency of 1/20 for 25sec. The solution was diluted to a final concentration of 10µg/mg and used for all enzyme assays.

Tyrosine Hydroxylase Activity Assay

Method was modified according to Nagatsu et al. (1992) and composed by the following ingredients: 40 µl phosphate buffer (0.1M, pH 6.6E); 50 µl ascorbic acid (0.1 M); 50 µl L-tyrosine (500 µM); 500 µl racemate 3,4-dihydroxyphenylalanine (100µM) dissolved in 0.1M HCl; 10µl protein solution (1mg/ml); 60 µl catalase (1mg/ml); 90 µl Ammonium tetrathiolate (1mM); 1,500 mM of l-ferric or racemic MPH was added to each tube and incubated for 45min at 37° C. The reaction was stopped with ethanol containing 20µl/ml 3,4-dihydroxybenzylamin (DHBs) or 0.2 M EDTA. The tyrosine was converted and treated with an alumina chromatoid Alox A column (Merck-Nagel, Dueren, Germany). Measurement of DOPAC and DHBs was done via high performance liquid chromatography. Statistical analysis was done using StatView and each experiment was repeated 6 times.

Monoamine Oxidase B Activity Assay

MAO-B activity was measured via a fluorescent based enzyme linked immunosorbent assay, as called Amplex® Red Monoamine Oxidase Assay Kit (Invitrogen, Karlsruhe, Germany). 50µl of protein solution was used for each experiment and 10µl of each dilutions in 100µM or racemate MPH doses (1-1000µM) was added. Procedure and measurement was done according to the manual. Statistical analysis was done using StatView and each experiment was repeated 6 times.

Conclusion

This exploratory investigation revealed in vitro pharmacological evidence for a potential difference between MPH racemate and isomers on dopaminergic enzyme activity. This finding might point to the therapeutic effects in the treatment of ADHD.

![Figure 1: Tyrosine hydroxylase (TH) activity after treatment with methylphenidate. Using a method according to Nagatsu, et al. (1) we investigated influence of different doses of MPH on in vitro TH activity in PC12 cell homogenates. Statistical analysis was done using StatView and each experiment was repeated 5 times.](image1)

![Figure 2: Monoamine Oxidase B (MAO-B) activity after treatment with methylphenidate (MPh). Protein solution of PC12 cells was incubated with different doses of MPH and afterwards measured via Amplex® Red Monoamine Oxidase Assay. Procedure and measurement was done according to the manual. Statistical analysis was done using StatView and each experiment was repeated 6 times.](image2)

![Figure 3: Alddeyde Dehydrogenase (ALDH) activity after treatment with methylphenidate (MPh). Protein solution of PC12 cells was incubated with different doses of MPH and ALDH activity was measured via microtiter plate reader. Absorbance was detected at 504nm for 10min. Statistical analysis was done using StatView and each experiment was repeated 3 times.](image3)