In vivo degradation of D-serine by D-amino acid oxidase and serine racemase in the central nervous system.

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Introduction

D-serine is the predominant D-amino acid in the mammalian central nervous system. D-serine is a transmitter acting as a co-agonist of N-methyl D aspartate (NMDA) receptors. D-serine has been implicated in several physiological functions such as brain development, learning and memory. It is also involved in psychiatric and neurological disorders, such as schizophrenia and neurodegenerative diseases like amyotrophic lateral sclerosis or Alzheimer's disease. The biochemical mechanisms responsible for D-serine degradation in the central nervous system (CNS) are still poorly understood. The enzyme D-Amino Acid Oxidase (DAAO) catalyzes D-serine oxidation into hydroxypurvate, but is only expressed in the hindbrain in adulthood[1]. In addition, serine racemase (SR), an enzyme expressed in the forebrain that synthesizes D-serine through L-Serine racemization, can also catalyze D-serine α,β-elimination into hydroxypyrurate in vitro[2]. The activity of these enzymes, however, has never been investigated in vivo. In this study, we investigated the effect of DAAO and SR inhibition on D-serine extracellular levels in cerebellum and cortex of adult male rats.

Methods

We have developed an enzymatic biosensor that specifically detects D-serine in the CNS [3]. The biosensor is made of a platinum wire coated with polyaniline, a screening polymer layer and with of the enzyme D-amino acid oxidase expressed from the yeast Rhodotorula gracilis (RgDAAO) and immobilized by cross-linking with poly(ethylene glycol) diglycidyl ether[4]. Enzymatic D-serine degradation produces \( \text{H}_2\text{O}_2 \), which is then oxidized at 500 mV vs Ag/AgCl. The response of the sensor is linear up to 500 µM D-serine, with a response time of ~2 s. Control sensors were prepared in the same way as D-serine sensors, except that RgDAAO was replaced by bovine serum albumin (BSA). To study the role of DAAO and SR, we recorded D-serine electrochemical signals at our biosensors in the brain of anesthetized rats with urethane. We also measured D-serine concentrations in brain homogenates ex vivo, to study serine racemase and α,β eliminase activity in the forebrain.
Results and Discussion

In basal conditions, D-serine extracellular level was undetectable (below 0.5 µM) in the cerebellum, and about 1.31 µM in the cortex. Three hours after an intraperitoneal injection of D-serine (1g/kg), D-serine extracellular concentration slowly increased by 0.9 ±0.39 µM in the cerebellum and 1.22 ±0.27 µM in the cortex. DAAO inhibition by intraperitoneal benzoate administration (2000 mg/kg) did not change D-serine basal levels in cerebellum and cortex. However, it dramatically increased D-serine penetration in the cerebellum after intraperitoneal D-serine injection (D-serine extracellular levels increased by 3.23 ±0.52 µM). By contrast, DAAO inhibition had no effect in the cortex. Conversely, SR was inhibited by intracerebroventricular injection of 0.5 µmol of Phenazine Ethosulfate (EtPhen). SR inhibition did not change D-serine basal extracellular levels. However, it facilitated D-serine penetration into the cortex (+50 % saline+D-serine, +110% EtPhen+D-serine), but not in the cerebellum.

Ex vivo, an injection of 300µM D-serine in a forebrain homogenate induced a step increase in D-serine concentration followed by a slow decrease indicating D-serine degradation. This decrease was blocked in the presence of Et-phen, suggesting D-serine degradation by SR-dependent α,β-elimination. Conversely, injection of 20 mM L-serine into the forebrain homogenate resulted in a slow increase in D-serine levels. This increase was again blocked by EtPhen, suggesting D-serine production by SR-dependent racemization. Therefore, both α,β-eliminase and racemase activities could be detected in the forebrain homogenates.

These results indicate that after systemic D-serine administration, most of D-serine crossing the blood-brain barrier is degraded by DAAO in the cerebellum and by SR in the cortex. In basal conditions (without D-serine administration) inhibition of these enzymes does not significantly change D-serine extracellular levels, suggesting that this transmitter has a slow turnover, i.e. low constitutive release and degradation. These results also confirm that serine racemase possesses a racemase and an α, β eliminase activity. The D-serine degradation mechanisms that are identified here can be the target of new pharmacological molecules aimed at modulating D-serine levels for treating diseases such as schizophrenia.

References