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Increase of monoamine oxidase-B activity in the brain of scrapie-infected hamsters

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In memory of Professor Dominique Dormont.

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ABSTRACT

In the present study, the purpose is to determine activities of monoamine oxidases (MAO) in the brain of 263K scrapie-infected hamsters during the development of this experimental prion disease. Indeed, MAO activity modifications which have already been related in aging and neurodegenerations is suspected to be involved in the neuron loss process by elevated hydrogen peroxide formation. Monoamine oxidase type A (MAO-A) and B (MAO-B) activities were followed in the brain at different stages of the disease. MAO-A activity did not change significantly during the evolution of the disease. However, concerning the MAO-B activity, a significant increase was observed from 50 days post-infection and through the course of the disease and reached 42.9 ± 5.3% at its ultimate stage. Regarding these results, MAO-B could be a potential therapeutic target then we have performed a pre-clinical treatment with irreversible (Selegiline or L-deprenyl™) or and reversible (MS-9510) MAO-B inhibitors used alone or in association with an anti-scrapie drug such as MS-8209, an amphotericin B derivative. Our results show that none of the MAO-B inhibitors used was able to delay the onset of the disease. Neither these MAO-B inhibitors nor R-NMDA inhibitors (MK-801) can enhance the effects of MS-8209. The present findings clearly indicate a significant increase of cerebral MAO-B activity in scrapie-infected hamsters. Furthermore, inhibitors of MAO-B do not have any curative or palliative effect on this experimental model indicating that the raise of this activity is probably more a consequence rather than a causal event of the neurodegenerative process.

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1. Introduction

Syrian hamster scrapie is a useful experimental model for the transmissible spongiform encephalopathies (TSEs) also named prion diseases which are neurodegenerative disorders of many mammalian species such as Creutzfeldt-Jakob disease in man, scrapie in sheep and more recently bovine spongiform encephalopathy (BSE) in cattle.

These fatal diseases are characterized by a long-incubation period. As the etiology of TSEs remains obscure, their diagnosis relies on their transmissibility and pathological and molecular hallmarks. Vacuolar degeneration of the neurophil and neuronal cell bodies is the most characteristic histopathological lesion; it is associated to gliosis with hypertrophy and possible proliferation of astrocytes.

At the molecular level, these disorders are characterized by the accumulation of an abnormal isoform (PrPres) of the host-encoded prion protein (PrP). PrPres is resistant to limited proteolysis and is derived from PrP post-translational modifications (Bolton et al., 1982). PrPres copurifies with the scrapie agent and is considered as the only component, or the major part of the agent (Prusiner, 1982; Weissmann, 1991). The transcriptional accumulation of glial fibrillary acidic protein (GFAP), a specific marker of astrocytes, has also been observed. GFAP and its mRNAs are overexpressed in both natural and experimental scrapie (Mackenzie, 1983; Lazarini et al., 1994).

Although these pathological and molecular features are of fundamental importance in relation to the neurological deficits, there are only limited data about the mechanisms underlying TSE

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neurodegenerative processes. Therefore, study of the free radical metabolism in the brain of scrapie-infected mice, revealed several enzymatic activity modifications (Lee et al., 1999a). Increases in catalase, monoamine oxidases (MAO) and a decrease in mitochondrial Mn-superoxide dismutase activities suggest that elevated oxygen free radical generation and lowered scavenging activity in mitochondria might cause the free radical damage to the brain.

MAO (EC1.4.3.4) are key enzymes in the deamination of monoamine neurotransmitters and neuromodulators. There are two isoenzymes, MAO-A and MAO-B, that differ in their selectivity for substrates, inhibitors and cellular localization, and that are encoded by two separated genes. MAO-A preferentially oxidases serotonine and norepinephrine (Westlund et al., 1988), whereas MAO-B preferentially oxidases histamine and β-phenylethylamine (β-PEA). Both isoforms oxidase dopamine (Konradi et al., 1988; Nakamura et al., 1990). It is well known that cerebral MAO-B activity increases with aging and during the development of several kind of neurodegenerative disorders such as Alzheimer, Parkinson and Huntington's diseases (Fowler et al., 1980; Mann et al., 1980; Jellinger and Riederer, 1984). This increase may be caused by the presence of MAO-B-rich reactive astrocytes and glial cells (Riederer et al., 1987) in response to neuronal degeneration.

The purpose of this study is to determine if TSEs exhibit the same MAO-B activity modifications as observed in the aging process and other neurodegenerations. Indeed, numerous similarities exist in these affections: neuropathological changes are restricted to the central nervous system (CNS) and classically consist of neuronal loss and astrogliosis. As observed in Alzheimer's disease, TSEs result in the accumulation of abnormally stable, potentially amyloidogenic protein (PrPres) that appear to play a central role in the pathogenesis of the disease. Then, we have measured the cerebral MAO-A and MAO-B activities in hamsters infected intracerebrally with the 263K scrapie agent to determine a possible modification of monoamine oxidase metabolism during scrapie and their implication in neurodegeneration and neuron loss. In a second time, we have tried to prevent the disease by a pre-clinical treatment using MAO-B inhibitors alone. In order to potentialise the effects of efficient anti-scrapie drugs such as MS-8209, an amphotericin B derivative (Demaimay et al., 1994, 1997; Adjou et al., 1995, 1996, 1999), MAO-B inhibitors were associated to the treatment.

2. Experimental procedures

2.1. Brain homogenates

Animals were killed by decapitation. Brains were rapidly removed, frozen in liquid nitrogen, and stored at −80 °C until use. Left cerebral hemispheres (cortex and cerebellum) were homogenized in phosphate buffer (100 mM, pH 7.2) using a Ribolyser (Hybaid). Homogenates were then suspended at 9% (wt/vol) with the same buffer for MAO activity determinations.

2.2. In vitro MAO activity determinations

MAO-A and MAO-B activities were determined radiochemically at 37 °C and pH 7.4 with [^{14}C] 5-HT and [^{14}C] β-PEA as substrates, respectively. The total volume of the mixture was 125 μl: 25 μl of diluted brain preparation, 75 μl of phosphate buffer, pH 7.4 (100 mM). The reactions were started by addition of 25 μl [^{14}C] 5-HT (340 μM, 74 KBq/μmol) or [^{14}C] β-PEA (8 μM, 1.48 MBq/μmol). After incubation at 37 °C, 5 min for MAO-A and 2 min for MAO-B, the reactions were stopped by addition of 100 μl of cold HCl (4N). Deaminated metabolites were extracted by vigorous shaking in 1.5 ml of a toluene/ethyl acetate mixture (1:1, vol/vol). Following extraction, 300 μl of the organic layer were poured into a vial containing 1 ml of scintillation solution (BCS-NA, Amersham, France) and were counted in a LKB 1209 Rackbeta liquid scintillation counter. All the assays were routinely triplicated.

2.3. Ex vivo MAO inhibition

Animals fasted for 12 h before treatment. MS-9510 was suspended in 50 μl of Tween 80 and then in 0.5 ml of aqueous solution of methyl cellulose 0.5%. A single oral dose of MS-9510 (50 mg kg^{-1}) or vehicle was administered. Groups of three animals were killed at different times after treatment.

2.4. Infection of animals

Outbreed, weanling female golden Syrian hamsters (age, 8 weeks; weight, 60–70 g) were obtained from the Centre d'élevage René Janvier (Le Genest-St-Isle, France). The animals received water and food *ad libitum*. Hamsters were injected intracerebrally with the 263K scrapie agent, a gift from H. Fraser, Edinburgh, United Kingdom (titer of the stock suspension, 2.2×10^{11} 50% lethal doses/g of brain). Fifty microliters of 1% (wt/vol) brain homogenate were injected into the right cerebral hemisphere. All experiments were carried out in accordance with the *European Communities Council Directive of 24 November 1986 (86/609/EEC).*

2.5. Drugs and reagents

MS-9510 ([2-hydroxy] ethylhydrazone of 4-benzyloxybenzaldehyde) was synthesized in the Mayoly Spindler laboratories as previously described (Bernard et al., 1995). L-deprenyl™ (Selegiline) and MK-801, a neuroprotective agent and a R-NMDA inhibitor, were purchased from Research Biochemicals International (France). MS-8209 is the *N*-methylglucamine salt of 1-deoxy-1-amino-4,6-*O*-benzylidene-D-fructosyl-AmB (Mayoly Spindler Laboratories, Chatou, France). Drugs were diluted in a 5% glucose (wt/vol) sterile solution.

2.6. Treatments

Animals were infected and treated with different drugs (MS-9510, MS-8209, L-deprenyl™, and MK-801) during the late stages of the incubation period of the disease. Drugs were administered either by the intraperitoneal route for MS-8209, L-deprenyl™, and MK-801 or by the oral route for MS-9510, 6 days a week, from day 30 to 50 of inoculation (experiment 1) or from day 30 post-infection until death (experiment 2). They were regularly monitored for the onset of clinical symptoms. Control groups were either untreated or injected intraperitoneally with the same volume of *N*-methylglucamine.

2.7. Statistical analysis

Student's *t*-test was used for finding significant differences between two means. p values <0.05 were considered significant. Two-way ANOVA followed by the evaluation of F value were determined with SPSS. Mann–Whitney *U*-test has been employed for the analysis of hamster survival times.

3. Results

3.1. Kinetic parameters

The experimental model of prion diseases used in this study was the golden Syrian hamster infected by the 263K scrapie agent. We have initially determined the kinetic parameters of hamster cerebral MAO-A and B. The K_m values, determined using the Lineweaver-Burk representation (data not shown), for hamster's cerebral MAO-A and -B, were equal to 170 and 4 μM, respectively. In all studies, care was taken to ensure that MAO-A and -B activities were linear with the enzyme concentration up to time period of the assay, and substrate deamination did not exceed 10%. In this case, substrates were in large excess and the reactions follow their initial rates.

3.2. Evolution of MAO activities during hamster scrapie

We have intracerebrally infected groups of three hamsters with the 263K scrapie agent and followed MAO activities in the left cerebral hemisphere during the development of the disease. Animals were also killed before injection (J0) and at the terminal stage of the disease (TS). As shown in Fig. 1A, the MAO-A activity did not change significantly during the evolution of the disease neither for 263K infected animals nor for those which had been intracerebrally injected by non-infectious brain homogenate (control). MAO-A activity observed at J0 was 94.7 ± 4.3 pmol of 5-HT oxidized/mg of brain min. A significant increase of the MAO-B activity (19.9 ± 4.3% as compared to J0) was observed from 50 days post-infection ($p = 0.043$, Fig. 1B). This augmentation reached 42.9 ± 5.3% at the ultimate stage of the disease ($p = 0.003$). In contrast, no significant variation of cerebral MAO-B could be observed in control animals whatever the date observed. MAO-B activity

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