

ANTIACETYLCHOLINESTERASE ACTIVITY OF CYCLOSPORINE - A COMPARISON OF SINGLE AND REPEATED ADMINISTRATION AND EFFECT OF 7-METHOXYTACRINE

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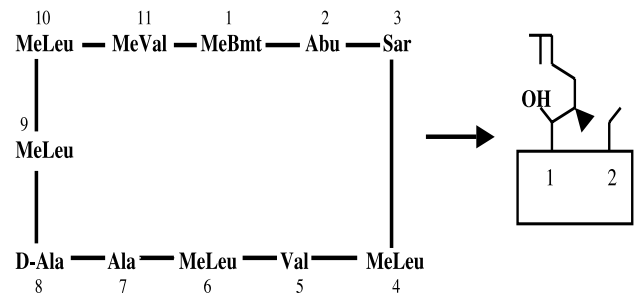
Summary: The aim of this work is a comparison of single and repeated peroral administration of cyclosporine (CsA) and the interaction of repeated administration of CsA and 7-methoxytacrine (MEOTA) on the activity of acetylcholinesterase (AChE) in the frontal cortex, hippocampus, septum, and basal ganglia in rats. Both single and repeated administration of CsA diminished the activity of AChE in the frontal cortex, septum and basal ganglia, while the enzyme activity in the hippocampus was diminished only in the case of repeated CsA, as well as repeated CsA + MEOTA administration. Repeated administration of CsA led to a further augmentation of anticholinesterase activity only in the frontal cortex and - in a lesser extent - in the basal ganglia. No augmentation of AChE activity was observed in the hippocampus and septum.

Key words: Acetylcholinesterase; Brain parts; Cyclosporine; 7-Methoxytacrine; Rat

Introduction

Cyclosporine (cyclosporine A, CsA) is a monopolar, cyclic polypeptide consisting of 11 amino acids (Fig.1). CsA mainly exhibits immunosuppressive activity. Moreover, it was experimentally as well as clinically demonstrated that CsA acts not only as an immunosuppressive agent but also as a drug which has beneficial effects on cells subjected to a variety of injurious conditions and was used as a useful agent for reducing cell damage (6, 14, 19). CsA, e.g., has been found to protect against dopaminergic depletion and to reduce cell death in animals models of stroke, cardiac arrest-induced and traumatic brain injury (12, 14). A membrane fluidizing effect of CsA enables to speculate on possibly increased penetration of some compounds through the cell membranes and organ barriers due to CsA (7). Indeed, CsA has been found to increase vincristine and vinblastine transport across the endothelial cell monolayer (3).

L-carnitine, a natural component of the mammalian tissue, is also capable to increase penetration of some chemical groups or drugs through biological barriers. We previously demonstrated an augmenting effect of repeated administration of L-carnitine on the anticholinesterase activity of 7-methoxytacrine (MEOTA) namely in the frontal cortex and septum (9). We therefore attempted to establish the ability of CsA to influence the antiacetylcholinesterase activity of 7-methoxytacrine (7-MEOTA).



MeBmt - 4-[(E)-2-butenyl]-4,4N-trimethyl-L-threonin; Abu - L-aminomáselná kyselina; Sar - sarkosin; MeLeu - N-methytleucin; Val - L-valin; Ala - L-alanin; D-Ala - D-alanin; MeVal - N-methylvalin

Fig. 1: Schematic representation of CsA molecule.

Material and Method

Male Wistar albino rats, weighing 200–230 g, were purchased from Biotest Ltd., Konárovice, Czech Republic. Animals were maintained in a air-conditioned room (22 ± 1 °C and 50 ± 10 % of relative humidity) with 12/12 day/night standard conditions and free access to standard chow and water. The directions of the Council of the European Communities (86/609/EEC) on animal care have been duly maintained. Handling of experimental animals was performed under the supervision of the local Ethical Committee.

Animals were divided into 4 groups with 6 in each. The study design was as follows:

1. Control group was administered only saline in a single dose in an amount of 0.1ml/100g p.o. and removal of the brain after 30 min.
2. Control group was administered of MEOTA in a single dose of 100 mg/kg i.m., removal of the brain after 30 min.
3. Administration of CsA in a single dose of 45 mg/kg p.o., removal of the brain after 30 min.
4. Repeated administration of CsA in three consecutive doses of 45 mg/kg p.o. separated by 24 hour intervals, removal of the brain 30 min after the last administration of the drug tested.
5. Repeated administration of CsA in three consecutive doses of 45 mg/kg p.o. separated by 24 hour intervals, application of MEOTA in a dose of 100 mg/kg i.m. after last CsA administration, removal of the brain after 30 min.

CsA was dissolved in olive oil, MEOTA was dissolved in saline, all doses tested were applied in an amount of 0.1 ml/100 g.

Animals were killed by decapitation and the following parts were prepared according to a previously described procedure: the frontal cortex, hippocampus, medial septum, and basal ganglia, respectively.

Acetylcholinesterase (AChE) activity in the homogenates (1:10) of the selected brain parts was determined using the method of Ellman et al. (4) as described elsewhere (1). Acetylthiocholine was used as the substrate and the results obtained were expressed as the number of nanomoles of the substrate hydrolyzed/min/100 mg wet weight tissue at 22 °C.

Acetylthiocholine was obtained from Lachema, Brno (Czech Republic). CsA was purchased from Sigma-Aldrich, St. Louis (USA).

Statistical significance was determined with the use of Student's test for independent samples and differences were considered significant when $p < 0.05$. Statistical analyses

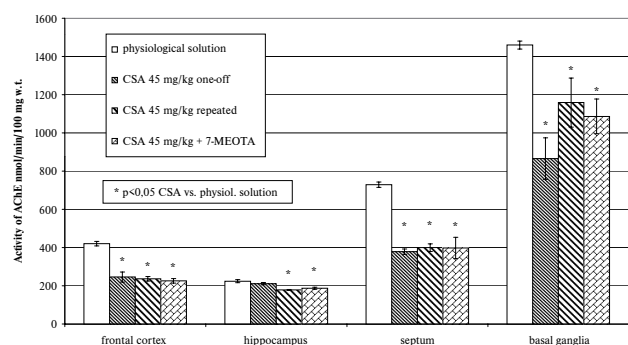


Fig. 2: Effect of CsA on AChE activity in the selected parts of the rat brain. (More detail description of control group 1 and experimental groups 3–5 see in text).

were performed on a PC using the programme Statistica 98 edition.

Results

CsA generally exerts inhibitory effect on the AChE activity in the brain parts chosen in comparison with the control (i.e., the „saline“) group 1. Moreover, no differences between the single and repeated administration were observed in the frontal cortex and septum, while the enzyme activity in the hippocampus was diminished only in the case of repeated (group 4) and combined CsA + MEOTA (group 5) administration. On the contrary, the single administration of CsA was more efficacious in comparison with repeated and combined ones in the basal ganglia, irrespective of statistical significance of groups 3–5 with control group 1 (Fig. 2).

A further augmentation of inhibitory activity of MEOTA following repeated administration of CsA was observed in the frontal cortex (see statistical significance between control „MEOTA“ group 2 and CsA + MEOTA group 5 in Fig. 3). Lesser extent of this augmentation (compare „MEOTA“ group 2 vs. group 4 and „MEOTA“ group vs. group 5) was observed in the basal ganglia. No augmentation was observed in the hippocampus and septum.

Discussion

Besides well-known side effects of CsA as, e.g., nephrotoxicity and hepatotoxicity, numerous side effects of central origin are observed (11, 14, 20). They include almost all categories, i.e., motoric, sensitive, affective, and cognitive functions. Some of them, such as confusion, seizure, spasticity, paresis or ataxia, could be attributed to acetylcholine system dysbalance. CsA exerts a protective effect on the density of muscarinic acetylcholine receptors following experimental brain ischemia (13) and also enhances a spontaneous acetylcholine release after a brief tetanus (16). Gaudry-Talarmain and Moulian (8) demonstrated an invol-

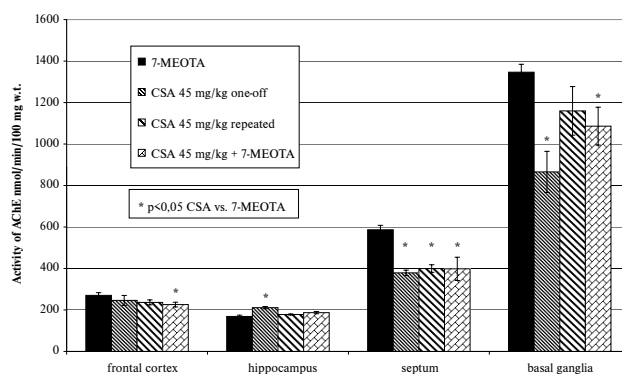


Fig. 3: A comparison of anticholinesterase activity of MEOTA and CsA. (More detail description of control group 2 and experimental group 3–5 see in text).

vement of CsA in the presynaptic mechanism of acetylcholine release in Torpedo synaptosomes. A prolongation of succinylcholine-induced neuromuscular blockade following CsA was also demonstrated (10). Famiglio et al. (5) observed epileptiform electroencephalographic (EEG) activity following CsA administered intraperitoneally in rats. Behavioural changes observed during abnormal EEG pattern were obviously subtle, animals were often still or slightly rocking. However, possible involvement of cholinergic mechanism in these findings is only a subject of speculation.

Borlongan et al. (2) proved the most relevant evidence concerning the interaction of CsA and the central cholinergic transmission in the brain. They described an enhancement of the septal choline acetyltransferase immunoreactivity in Wistar rats. The involvement of the septal region in the central effect of CsA is in good agreement with our observation.

We demonstrated at least the same (in case of the frontal cortex and septum), or even higher (in case of the basal ganglia) inhibition efficacy of CsA in comparison with MEOTA. Moreover, the dose of MEOTA used in our experiments is relatively high, it corresponds to LD₂₀ (1). The enhancement of the anticholinesterase activity of MEOTA following repeated administration of L-carnitine has been previously demonstrated. L-carnitine augmented this activity in the frontal cortex and septum, and - in case of higher doses used - also in the basal ganglia (9). On the contrary, the extent of the augmentation of anticholinesterase activity of MEOTA following CsA was less pronounced. It was apparent only in the frontal cortex, and - under some circumstances - in the basal ganglia. This finding confirms previously published results supporting the higher sensitivity of AChE in the frontal cortex to the effect of tacrine and its derivatives (9). However, the above mentioned differences between L-carnitine and CsA in relation to the anticholinesterase activity of MEOTA suggest different mechanisms of this interaction.

According to the most accepted opinion CsA acts through binding with the immunophilin cyclophilin, the complex CsA/cyclophilin inhibits calcineurin (16, 17). Calcineurin has been shown to be localized throughout the brain including septum and hippocampus and it is important for nitric oxide (NO) metabolism and nuclear import of transcription factors (2, 16). There is also evidence about the involvement of calcineurin in the synthesis of AChE (18). The possible link CsA - calcineurin - synthesis of AChE claims to explain the above described changes in the AChE activity after CsA.

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