Effect of 7-Methoxytacrine and L-Carnitine on the Activity of Choline Acetyltransferase

J. BAJGAR[1], F. SKOPEC[2], J. HERINK[1], J. PATOČKA[1] AND J. KVĚTINA[3]

1 *Military Medical Academy, Hradec Králové, Czech Republic*
2 *Medical Faculty, Charles University, Hradec Králové, Czech Republic*
3 *Institute of Experimental Biopharmacy, PRO.MED.CS and AV CR, Hradec Králové, Czech Republic*

Abstract. Changes of choline acetyltransferase (ChAT) activity in the hippocampus and the basal ganglia were studied in rats treated i.p. with L-carnitine (CRT) and 7-methoxytacrine (7-MEOTA) (i.m.) separately or 3-days treated with L-carnitine and then with one administration of 7-MEOTA. Both compounds increased ChAT activity when administered separately. 3-day treatment of CRT followed by administration of 7-MEOTA normalized ChAT activity.

Key words: Hippocampus — Basal ganglia — Rat — Choline acetyltransferase

Introduction

Alzheimer's disease (AD) is a complex disease with wide implications. It is a major cause of dementia affecting more than 1% of population aged more than 60, and 21% of those aged 85-89 and 39 % of those over 90 (Brufani at al. 1997; Mc Geer and Mc Geer 1995). The disease is characterized by neuronal loss, synaptic damage, neurofibrilary tangles and neuritic and vascular plaques. At the cellular level, AD is associated with reduced levels of synaptic acetylcholine and other neurotransmitters.Choline acetyltransferase (ChAT, EC 2.3.1.6) and acetylcholinesterase (AChE, EC 3.1.1.7) are reduced, as is the number of nicotinic acetylcholine receptors when compared with age-matched controls (Mc Geer and Mc Geer 1995).Thus, AD has been shown to correlate with a reduction of markers for brain cholinergic transmission.This led to treatment attempts with cholinomimetics including cholinesterase inhibitors. A reversible inhibitor of cholinesterases, tacrin (1,2,3,4-tetrahydro-9-aminoacridine) belongs to a group of drugs which were reported to be useful in the therapy of Alzheimer's disease, as antidotes against intoxication with anticholinergics, in tardive dyskinesias and others. However, its toxicity is relatively high and side effects and hepatotoxicity have been observed. 7-methoxyderivative of

Correspondence to: Doc. MUDr. J. Bajgar, DrSc., Military Medical Academy, Třebešská 1575, 500 01 Hradec Králové, Czech Republic. E-mail: bajgar@pmfhk.cz

tacrin, 7-MEOTA has a weaker toxicity in comparison with tacrin, and a better therapeutic efficacy.

It can be characterized as a reversible inhibitor of cholinesterases *in vitro* and *in vivo* (Bajgar et al. 1994). The natural component of the mammalian tissue L-carnitine (CRT) is known to increase penetration of some drugs or chemical groups through biological barriers. Moreover, both drugs enhance cholinergic transmission in the brain (Bajgar et al 1994; Říčný et al 1992). It was demonstrated previously that 7-MEOTA inhibits AChE in different parts of the brain and also its metabolites are inhibitors of the brain AChE *in vivo* (Bajgar et al. 1994). However, it is not known if the other important marker of cholinergic nerve transmission – ChAT – is influenced by these conditions. The aim of the present study was to determine ChAT activity in selected parts of the rat brain following administration of 7-MEOTA and to compare it with the activities in animals pretreated with CRT before 7-MEOTA administration

Materials and Methods

Female Wistar rats (VELAZ Prague, Czech Republic), weighing 160-200 g were used The animals were divided into groups of 6 animals each The changes in the activity of ChAT were determined in homogenates (1 10, distilled water) of the rat brain hippocampus (H) and basal ganglia (BG) Activities of ChAT were determined according to Tuček (1973), and were expressed as pmol/min/mg wet weight tissue Doses of 0 1 ml/100 g of saline (i p) were administered daily for 3 consecutive days to control group The third day after the injection of saline, 7-MEOTA in a dose of 100 mg/kg (i m) was administered 30 minutes after the saline injection A repeated administration of different doses of CRT (100, 200, 250, 300, and 400 mg/kg, i p) in three consecutive injections separated by 24 hour intervals was used in experimental animals A separate experimental group was used for each dose of CRT chosen On the third day of experiments, 7-MEOTA in a dose of 100 mg/kg i m was given to each experimental group 30 minutes after to the third CRT injection Handling of experimental animals was performed under supervision of the Ethics Committee of the Medical Faculty of Charles University and the Military Medical Academy, Hradec Králové, Czech Republic

Results

Control activities of the ChAT were higher in BG in comparison with H. Administration of CRT caused an increase of ChAT activity in both tissues studied. Following 7-MEOTA administration, an increase of ChAT activity in the parts of the brain was also observed Thus, both, 7-MEOTA and CRT, when administered separately, enhanced ChAT activity (Table 1, control 2 and 3) On the contrary, following simultaneous administration of CRT and 7-MEOTA no changes in ChAT activity were observed (Table 1, CRT 250 and 400). Statistically significant ($p \leq 0.05$) differences are indicated in Table 1.

Table 1. Changes in ChAT activity (pmol/min/mg wet weight tissue)

Group	H	BG
control 1	66.0 ± 5.6	155.4 ± 20.0
Bcontrol 2 CRT 250	98.1 ± 5.8	197.5 ± 22.7
control 2 CRT 400	101.4 ± 5.9	181.0 ± 18.5
control 3	93.5 ± 6.1	172.5 ± 16.9
CRT 250	67.3 ± 4.9	140.1 ± 20.1
CRT 400	70.7 ± 5.7	170.3 ± 21.2

The results are means ± S.D. Control 1, CRT 250 + 7-MEOTA, CRT 400 + 7-MEOTA are statistically different from the remaining groups for both brain tissues investigated (H + BG). Control 1: saline only, control 2: CRT in two different doses only, control 3: 7-MEOTA only.

Discussion

Cholinergic neurons contain several proteins which are related to the metabolism of the cholinergic neurotransmitter acetylcholine. These proteins include the high-affinity carrier for choline located in the nerve terminal presynaptic membrane (Yamamura and Snyder 1973), ChAT located in the cytoplasm and the outer surface of synaptic vesicles (Wu and Hersh 1994), vesicular carrier for acetylcholine located in the membrane of synaptic vesicle (Parsons et al. 1993) and AChE, located mostly on postsynaptic membrane (Massoulié et al. 1993). ChAT is a more specific marker of cholinergic nerve transmission than AChE. Among these proteins, changes of two (ChAT and AChE) were described. As for the influence of 7-MEOTA or CRT on these enzymes, AChE was studied in more detail. Following 7-MEOTA administration AChE was shown to be inhibited (de Sarno et al. 1989; Sherman and Messamore 1989; Bajgar et al. 1994). The inhibition was more expressed in H in comparison with BG. This would support the hypothesis on the importance of this structure for the action of 7-MEOTA. The stronger inhibition of AChE following 7-MEOTA administration in animals pretreated 3 days with CRT suggested an increase of the penetration of 7-MEOTA through the blood-brain barrier. The most marked effect was observed after CRT dose of 250 mg/kg. CRT alone did not influence AChE activity (Bajgar et al. 1998).

The differences in the inhibition of AChE in the brain structures observed could suggest a various importance of them for the action of 7-MEOTA and CRT. The higher degree of inhibition following CRT pretreatment could suggest either a change in the blood-brain barrier by CRT or influencing of 7-MEOTA inhibition efficacy by the same drug.

There are no similar data for ChAT. Normal activities of ChAT in the brain tissues studied are in good agreement with our previous studies (Bajgar et al. 1985) and those reported in literature (Frick et al. 1996). It is known that ChAT activity is influenced by many factors like chemicals (Bajgar et al. 1985; Wu and Hersh 1994; Frick et al. 1996), brain lesions (Herink et al. 1975), hypertension (Bajgar

et al 1979, Brezenoff and Giuliano 1982) hormonal action (Tuček et al 1976) etc It appears from our results that also the compounds studied (i e CRT and 7-MEOTA) are able to influence ChAT activity *in vivo* However, the mechanisms of this influencing are unclear but possibly reflect a compensatory mechanism to counteract the drugs used

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