EFFECT OF ACETYL-L-CARNITINE ON THE ANTICHOLINESTERASE ACTIVITY OF 7-METHOXYTACRINE IN VIVO

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Summary

The aim of this work is a comparison of repeated peroral (p.o.) administration, i.e., three consecutive doses separated by 24-hour intervals of acetyl-L-carnitine (ALC) and L-carnitine (CRT) on the antiacetylcholinesterase potency of 7-methoxytacrine (MEOTA) given intramuscularly (i.m.) in a dose of 100 mg/kg. Changes of acetylcholinesterase activity were determined in the frontal cortex, hippocampus, medial septum, and basal ganglia in rats.

Repeated administration of CRT in doses of 100, 250, 300 and 400 mg/kg augmented the anticholinesterase activity of MEOTA in the frontal cortex and septum, higher doses of 250, 300 and 400 mg/kg also increased inhibitory activity of MEOTA in the basal ganglia, while none of doses tested did not influence the inhibitory activity of MEOTA in the hippocampus.

Repeated administration of ALC in doses of 100, 200, 250, 300 and 400 mg/kg did not influence anticholinesterase activity of MEOTA in the frontal cortex, hippocampus and basal ganglia, moreover, the inhibitory effect of MEOTA in the septum was abolished.

The results obtained suggest different mechanisms of the mutual interaction of CRT with MEOTA on the one hand, and ALC with the same drug on the other hand.

Introduction

7-methoxytacrine (MEOTA) is a relatively moderate inhibitor of cholinesterases *in vitro* as well as *in vivo* conditions (1). MEOTA has in comparison with its parent drug tacrine a lower degree of undesirable effects, especially of hepatotoxicity (2). Inhibition of acetylcholinesterase (AChE, EC 3.1.1.7) is used as the most important marker characterizing the action of these compounds (3).

L-carnitine (CRT), a natural component of the mammalian tissue, is capable to increase penetration of some chemical groups or drugs through biological barriers (4, 5). Moreover, CRT enhances a cholinergic transmission in the brain (6). Acetyl-L--carnitine (ALC) is an ester of CRT, and it is synthesized in the brain, liver a kidney. ALC facilitates the uptake of acetyl CoA into mitochondria during fatty acid oxidation, enhances acetylcholine production, and stimulates protein and membrane phospholipid synthesis (7). ALC possesses in comparison with CRT a higher degree of bioavailability and lipo-solubility (8).

The following benefit could be expected from simultaneous administration of MEOTA and CRT, maybe also ALC: A higher penetration of MEOTA through the blood-brain- barrier towards the target sites in the central nervous system or a direct interaction of drugs tested on the active sites.

The aim of this work is a comparison of repeated peroral (p.o.) administration of ALC on the antiacetylcholinesterase potency of MEOTA in the rat brain.

Material and methods

Chemicals:

7-methoxytacrine (Purkyně Military Medical Academy, Hradec Králové),
L-carnitine hydrochloride (Sigma-Aldrich, St. Louis, USA),
acetyl-L-carnitine hydrochloride (Sigma-Aldrich, St. Louis, USA),
acetylthiocholine was obtained from Lachema, Brno.

Animals:

96 male Wistar rats weighing 180–220 g from the conventional breed VELAZ (Prague). Animals were divided into groups of 6 each. Handling of animals were performed under supervision of the Ethic Committee of the Military Medical Academy and Medical Faculty of Charles University, Hradec Králové.

Working procedure:

Doses of 0,1 ml/100 g of saline were administered p.o. daily for 3 consecutive days to control group 1. Animals of control group 2 received the same amount of saline for 3 consecutive days. On the third day MEOTA in a dose of 100 mg/kg was injected i.m. 30 min following last saline administration.

Three applications of various doses of CRT (100, 200, 300 and 400 mg/kg, p.o.) separated by 24 hour intervals were performed in animals of the four experimental groups. On the third day of experiments, MEOTA in a dose of 100 mg/kg was injected 30 min after last CRT administration. The same schedule was used in a case of ALC administration in doses of 100, 200, 250, 300 and 400 mg/kg.

The animals were killed by exsanguination 30 min after last administration, the brains were removed and following brain parts were prepared: the frontal cortex, hippocampus, medial septum and basal ganglia. AChE activity in homogenates (1.10) of the brain parts was determined according to modified Ellman's method (9) and expressed as nmol of substrate (acetylthiocholine) hydrolyzed/ /min/100 mg of the wet brain tissue.

Statistics:

Statistical significance was determined by the use of Student's test and differences were consider significant when $p < 0,05$. Statistical evaluation was performed with relevant computer programmes (Hewlett Packard 9830A).

Results and conclusions

Single administration of MEOTA, i.e. without previous premedication with CRT or ALC, led to a decrease in AChE activity in the sequence the frontal cortex (the most marked decline) > hippocampus > septum. On the contrary, AChE activity was not influenced in the basal ganglia (Table l, control 2). ALC itself given p.o. did not influence AChE activity in the brain parts studied (Table 1, ALC groups with different doses of ALC).

In case of pretreatment with CRT, a further augmentation of inhibitory activity of MEOTA in comparison with the both control l (only saline) and control 2 (saline + MEOTA) groups was observed (Table 2). The involvement not only of the frontal cortex, hipocampus and septum, but also basal ganglia in this effect of CRT is noticeable.

Table l

Effect of ALC on activity of AChE in the selected brain structures control 1: saline only, control 2: saline + MEOTA, experimental groups: only ALC in three p.o. administration separated by 24 hour intervals.

Group	FC	H	S	BG
control 1 (saline only)	421,5	222,7	746,0	1484,2
control 2 (saline + MEOTA)	270,2*	168,5*	586,6*	1346,3
ALC 100	436,7	221,0	713,0	1450,0
ALC 200	451,2	229,3	715,5	1470,0
ALC 250	435,2	216,3	720,7	1455,0
ALC 300	450,3	230,4	709,7	1450,0
ALC 400	438,3	228,7	715,7	1459,0

FC - frontal cortex, H - hippocampus, S - septum, BG - basal ganglia, * effect statistically significant in comparison with control 1.

Table 2

Effect of p.o. administration of CRT on anticholinesterase activity of MEOTA control: saline + MEOTA, * effect statistically significant in comparison with control group

Group	FC	H	S	BG
control (saline + MEOTA)	270,2	168,5	586,6	1346,3
CRT 100 + MEOTA	183,7*	190,0	426,5*	1057,5
CRT 250 + MEOTA	167,5*	174,2	360,2*	642,3*
CRT 300 + MEOTA	157,8*	161,3	320,2*	490,2*
CRT 400 + MEOTA	172,2*	175,8	382,3*	525,5*

Table 3

Effect of p.o. administration of ALC on anticholinesterase activity of MEOTA control 1: saline only, control 2: saline + MEOTA, * effect statistically significant in comparison with control 2 (i.e. interaction of repeated administration ALC with MEOTA in the septal area).

Group	FC	H	S	BG
control 1 (saline only)	421,5	222,7	746,0	1484,2
control 2 (saline + MEOTA)	270,2	168,5	586,6	1346,3
ALC 100 + MEOTA	271,0	175,0	705,3*	1468,0
ALC 200 + MEOTA	285,0	181,0	700,2*	1483,0
ALC 250 + MEOTA	283,5	190,7	705,3*	1455,0
ALC 300 + MEOTA	318,4	190,0	692,2*	1445,0
ALC 400 + MEOTA	290,5	192,2	691,6*	1443,0

In case of pretreatment with ALC, a quite different effect relating to inhibitory activity of MEOTA was observed. Anticholinesterase activity of MEOTA was not influenced by ALC pretreatment in the frontal cortex and hippocampus. Moreover, the tendency to abolish the inhibitory activity of MEOTA was observed in the basal ganglia. This abolition was

statistically significant in the septal area (Table 3).

The results obtained confirm the hypothesis concerning the possibility of synergy of MEOTA and CRT on the active sites in the observed parts of the brain. However, different mechanisms of interaction of MEOTA with ALC will be presumed in comparison with CRT premedication.

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