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Inhibition of choline acetyltransferase by excitatory amino acids as a possible mechanism for cholinergic dysfunction in the central nervous system

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Abstract

Choline acetyltransferase (ChAT) activity was reduced by more than 85% in cultured retina cells after 16 h treatment with 150 μM kainate ($T_{1/2}$: 3.5 h). Glutamate, AMPA and quisqualate also inhibited the enzyme in equivalent proportion. Cell lesion measured by lactate dehydrogenase (LDH) release, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide - thiazolyl blue (MTT) reduction and microscopic observation was not detected even after 48 h with kainate. Other retina neurochemical markers were not affected by kainate and full recovery of the enzyme was achieved 9 days after kainate removal. Moreover, hemicolinium-3 sensitive choline uptake and hemicolinium-3 binding sites were maintained intact after kainate treatment. The immunoblot and immunohistochemical analysis of the enzyme revealed that ChAT molecules were maintained in cholinergic neurons. The use of antagonists showed that ionotropic and group 1 metabotropic receptors mediated the effect of glutamate on ChAT inhibition, in a calcium dependent manner. The quisqualate mediated ChAT inhibition and part of the kainate effect (30%) was prevented by 5 mM N^G-nitro-L-arginine methyl ester (L-NAME). Veratridine (3μM) also reduced ChAT by a Ca^{2+} dependent, but glutamate independent mechanism and was prevented by 1μM tetrodotoxin.

Keywords: choline acetyltransferase inhibition, glutamate receptors.

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Cholinergic dysfunctions are thought to be involved in selective disorders of the central nervous system. For instance, some features of Alzheimer's disease are attributed to cholinergic dysfunctions in areas of the brain related to cognition (Selkoe 1997; Auld *et al*. 1998; Cummings and Back 1998; Smith 1998). Also, acute traumatic stress may lead to post-traumatic stress disorder that seems to reflect changes in cholinergic properties of the brain (Milner *et al*. 1994; Kaufer *et al*. 1998). However, the difficulty in assessing the early stages of functional cholinergic alterations, as a result of environmental modifications, rests on the fact that central cholinergic systems are basically constituted of populations of interneurons and projection neurons of which terminals are topographically distant from the soma. Therefore, it is difficult to have full information about the functional integrity of the whole system based on the analysis of the different cholinergic markers.

The retina cholinergic system is entirely contained within tissue. All cholinergic cells in the retina are amacrine neurons the cell bodies of which are located in the inner portion of the inner nuclear layer and also displaced to the ganglion cell layer. These cells project their neurites to the inner plexiform layer where they form two, well defined,

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Abbreviations used: BIM, benzydolylmaleimide; ChAT, choline acetyltransferase; EAAs, excitatory amino acids; hemicolinium-3, (HC-3); KN-62, {1-[*N*,*O-bis*-(5-isoquinolinesulfonyl)-*N*-methyl-L-tyrosyl]-4-phenylpiperozine}; L-NAME, N^G-nitro-L-arginine-methyl-ester; LDH, lactate dehydrogenase; LY294002, (2-(4-morpholinyl)-8-phenyl-4*H*-1-benzopyran-4-one); MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide – thiazolyl blue; PAGE, polyacrylamide gel electrophoresis; PD098059, 2-(2-amino-3-methoxyphenyl)-4*H*-1-benzopyran-4-one; PMA, phorbol 12-myristate 13-acetate; SB202190, [4-(4-fluorophenyl)-2-(4-hydroxyphenyl)-5-(4-pyridyl)-1*H*-imidazole – FHPI].

