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Research Report

Chronic treatment with amyloid β_{1-42} inhibits non-cholinergic high-affinity choline transport in NG108-15 cells through protein kinase C signaling

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Abstract

We investigated the influence of the amyloid-β-peptide$_{1-42}$ on hemicholinum-3-sensitive high-affinity choline uptake in NG108-15 cells. RT-PCR analysis revealed the presence of mRNA for a choline transporter-like protein but not for cholinergic high-affinity choline transporter. Differentiation of cells increased both hemicholinum-3-sensitive choline uptake and high-affinity hemicholinium-3 binding. This transport was not influenced by tenfold excess of carnitine. Continuous presence of submicromolar concentrations of amyloid-β-peptide$_{1-42}$ during differentiation resulted in a decrease of both choline uptake and hemicholinium-3 binding. These effects were not present when amyloid-β-peptide$_{1-42}$ was added 5 min prior to measurements. Neither differentiation nor amyloid-β-peptide$_{1-42}$ treatment changed levels of choline transporter-like protein mRNA. Protein kinase C inhibition by staurosporine or its inactivation by continuous presence of tetradecanoyl phorbol acetate prevented the inhibitory effect of amyloid-β-peptide$_{1-42}$ treatment on choline uptake. Activation of protein kinase C by tetradecanoyl phorbol acetate during measurement had inhibitory effect on choline uptake in control but not amyloid-β-peptide$_{1-42}$-treated cells. The concentration of amyloid-β-peptide$_{1-42}$ maximally effective on hemicholinium-3-sensitive choline uptake had no effect on cell growth, oxidative activity, membrane integrity, number of surface muscarinic receptors, caspase-3 and -8 activities, or uptake of deoxyglucose. Results demonstrate that long-term treatment with non-toxic concentrations of amyloid-β-peptide$_{1-42}$ downregulates choline uptake presumably mediated by a choline transporter-like protein through activation of protein kinase C signaling. The decrease of choline uptake may have relevance to the pathogenesis of Alzheimer's disease.

Theme: Disorders of the nervous system
Topic: Degenerative disease: Alzheimer's beta amyloid

Keywords: β-amyloid; High-affinity choline transport; Hemicholinium-3 binding; Choline transporter 1; Choline transporter-like protein; Protein kinase C

1. Introduction

It is generally accepted that malfunction in the metabolism of amyloid precursor protein (APP) plays a fundamental role in the pathogenesis of Alzheimer's disease [28,45]. A large body of evidence indicates that an increased production or decreased clearance of Aβ fragments or a combination of both processes leads to the development of Alzheimer's disease [6,45]. Most recent findings are consistent with a notion that soluble oligomeric Aβ fragments and not insoluble amyloid plaques are involved in the initiation and progression of the disease [19,25,46]. Probably, the most toxic species of Aβ fragments is that comprising 42 amino acid residues (Aβ_{1-42}). In spite of a substantial experimental effort, however, there is no general consensus as to what constitutes the early pathogenic influence of Aβ_{1-42} on neuronal functions [20].

Abbreviations: Amyloid β-peptide1–42, Aβ_{1-42}; Amyloid Precursor Protein, APP; Hemicholinum-3, HC-3; Choline Transporter-like Protein 1, CTL1; High-affinity choline transporter 1, ChT1; N-methylscopolamine, NMS; Protein Kinase C, PKC; Tetradecanoyl Phorbol Acetate, TPA

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Impairment of cholinergic neurons in basal forebrain innervating cerebral cortex and hippocampus is invariably found in Alzheimer's brains at autopsy. It is not known whether this damage is due to a general neuronal degeneration occurring at a late stage of the disease or whether cholinergic neurons are more sensitive to Aβ toxicity and involved in the pathogenesis of the disease [3,11,18]. Several aspects of cholinergic neurotransmission have been shown to be targets of physiologically relevant concentrations of Aβ [2]. We have observed in previous experiments that submicromolar concentrations of $A\beta_{1-42}$ present in the culture medium during differentiation of the cholinergic cell line NG108–15 [23,24] inhibited N-type calcium channels [31]. The differentiation also increased transcription of genes embedded in the cholinergic gene locus and enhanced general neuronal phenotype [7,10,12,13,15].

These findings led us to investigate the influence of $A\beta_{1-42}$ on a high-affinity choline uptake in NG108-15 cells. Using RT-PCR analysis we found that these cells do not express the specific cholinergic high-affinity choline transporter ChT1 [1,40,41]. However, we have identified expression of a hemicholinium-3-sensitive (HC-3) choline transporter-like protein (CTL1) that is similar to ChT1 [39]. CTL1 is widely expressed in the brain and its overexpression in neuroblastoma cells has been shown to increase choline uptake [48]. We demonstrate that chronic but not acute treatment with non-toxic concentrations of $A\beta_{1-42}$ diminishes both HC-3-sensitive choline uptake and high-affinity ^{3}H-HC-3 binding occurs downstream of gene transcription and involves protein kinase C signaling.

2. Materials and methods

2.1. Cell culture

NG108-15 cells were cultured as described [11]. Briefly, they were grown in Dulbecco's modified Eagle's medium containing 5% non-inactivated foetal calf serum, 1% HAT supplement (Sigma; containing hypoxanthine, aminopterin and thymidine), 3 μmol/l glycine, 2 mmol/l glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin, under an atmosphere of 5% CO_2/95% humidified air at 37 °C. For hemicholinium-3 (HC-3) binding and choline uptake experiments they were seeded on 10 cm Petri dishes at a density of 200,000 cells per dish in 10 ml of supplemented DMEM. For all other experiments, they were seeded in 24-well plates at a density 20,000 cells per well in 2 ml of supplemented DMEM. Drugs were added the next day as indicated and the cells were grown in their presence for 4–5 days without change of the medium. Differentiation was induced by 0.2 mmol/l dibutyryl cAMP and 100 nmol/l dexamethasone. $A\beta_{1-42}$ was dissolved in redestilled water at a concentration of 100 μmol/l at room temperature and stored frozen in aliquots before use. It has been demonstrated that formations of the toxic soluble oligomers is reached within a few minutes and ageing does not increase its toxicity [14].

2.2. ^{3}H-Hemicholinium-3 binding

Medium was removed and cells were released into 6 ml of Krebs–HEPES buffer (final concentrations in mmol/l: NaCl 138, KCl 4, $CaCl_2$ 1.3, $MgCl_2$ 1.2, NaH_2PO_4 1.2, glucose 11, HEPES 10, pH 7.4) and collected by centrifugation (5 min at 200 × g). Cell pellets were resuspended in fresh Krebs–HEPES buffer (300–500 μl per one Petri dish) and aliquots (50 μl) of the cell suspension were added in triplicate to eppendorf test tubes containing 50 μl of the buffer with labeled HC-3 and incubated 30 min at 37 °C. At the end of incubation, they were chilled on ice and pelleted for 5 min at 2000 g in a refrigerated centrifuge. Supernatants were carefully removed, the cell pellets were surface washed with 200 μl of ice-cold Krebs–HEPES buffer and centrifuged again for 2 min at 2000 × g. Supernatants were discarded and cell pellets were dissolved in 100 μl aliquots of 1 mol/l sodium hydroxide. 60 μl aliquots were used for scintillation counting and 20 μl aliquots for protein determination. Non-specific binding was measured in the presence of 20 μmol/l unlabeled HC-3 in single point determinations or 10 mmol/l choline in kinetic experiments. The displaceable binding of HC-3 ranged between about 40% of total binding in low concentrations and 10% in high concentrations of tracer. For this reason, the single point measurements were performed using 5–10 nmol/l of ^{3}H-HC-3 and the results are expressed as percent of controls in individual experiments.

2.3. ^{3}H-Choline uptake

Cells were treated and harvested as described for HC-3 binding. The cell pellet was resuspended in 10 ml of Krebs–HEPES buffer and incubated for 30 min at 37 °C in a Petri dish to deplete endogenous choline. Cells were collected again, resuspended in Krebs–HEPES buffer (300–500 μl per one Petri dish) and 50 μl aliquots of cell suspension were added to Eppendorf test tubes in ice. 50 μl aliquots of buffer with ^{3}H-choline were added and samples were incubated in most cases (see text to figures) for 4 min at 37 °C. Incubation was stopped by transferring samples to an ice bath and adding 500 μl of ice cold Krebs–HEPES buffer containing 10 μmol/l HC-3. The samples were than processed as described for HC-3 binding. Non-specific uptake of choline was determined in parallel samples that contained 10 μmol/l HC-3 during the uptake period.

2.4. ^{3}H-NMS binding

Medium was removed and l ml of Krebs–HEPES buffer containing 2 nmol/l ^{3}H-NMS or ^{3}H-NMS plus 5 μmol/l atropine was added into the wells in triplicate. The plates