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BRAIN RESEARCH

Research Report

Determination of high-affinity choline uptake (HACU) and choline acetyltransferase (ChAT) activity in the same population of cultured cells

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ARTICLE INFO

Article history:
Accepted 22 July 2009
Available online 4 August 2009

Keywords:
Acetylcholine
Choline uptake
ChAT
Differentiation
Enzyme assay
HACU
Neuronal cells
Neurodegenerative disorders
Tissue culture

ABSTRACT

Cholinergic neurons are a major constituent of the mammalian central nervous system. Acetylcholine, the neurotransmitter used by cholinergic neurons, is synthesized from choline and acetyl CoA by the enzymatic action of choline acetyltransferase (ChAT). The transport of choline into the cholinergic neurons, which results in synthesis of ACh, is hemicholinium-sensitive and is referred to as high-affinity choline uptake (HACU). Thus, the formation of acetylcholine in cholinergic neurons largely depends on both the levels of choline being transported into the cells from the extracellular space and the activity of ChAT. Several methods were described previously to measure HACU and ChAT simultaneously in synaptosomes, but the same for cultured cells is lacking. We describe a procedure to measure HACU and ChAT at the same time in cultured cells by simple techniques employing radionuclides. In this procedure, we determined quantitatively hemicholinium-sensitive choline uptake and ChAT enzyme activity in a small number of differentiated human neuroblastoma (SK-N-SH) cells. We also determined the kinetics of choline uptake in the SK-N-SH cells. We believe that these simple methods can be used for neurochemical and drug discovery studies in several models of neurodegenerative disorders including Alzheimer's disease.

1. Introduction

Acetylcholine is the neurotransmitter in cholinergic neurons and is synthesized in the nerve terminal following transport of choline from the extracellular space. Choline is transported into the cell by the high-affinity choline transporter (Apparsundaram et al., 2000) where it reacts with acetyl CoA in the presence of the enzyme choline acetyltransferase (ChAT) to form acetylcholine. High-affinity choline uptake into cholinergic cells can be completely blocked by hemicholinium-3 and is referred to as high-affinity choline uptake (HACU) (Simon and Kuhar, 1975). Loss of cholinergic neurons is pathognomonic in several neurodegenerative disorders like Alzheimer's disease (AD) (Ginsberg et al., 2006). One of the important modes of treatment in AD is to restore cholinergic transmission by preventing the degradation of

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doi:10.1016/j.brainres.2009.07.096

acetylcholine by acetylcholinesterase (Birks et al., 2009). Indeed, there are four FDA-approved cholinesterase inhibitors (ChEIs) and a partial NMDA receptor antagonist, memantine, being used for the treatment of AD. However, their effects on HACU are unknown. In addition to ChEIs other strategies, such as estrogen replacement therapy, neuroprotective compounds and antioxidants are being investigated for the prevention and treatment of AD (Lahiri et al., 2003). In this context, the ability of estrogen to increase HACU activity in cultured basal forebrain cholinergic neurons assumes significance (Bennett et al., 2009). Our goal is to test whether ChEIs, both FDA-approved and the newer drugs, have effects on choline uptake and/or ChAT, and for this purpose we developed a procedure to measure HACU and ChAT at the same time in neuronally differentiated cultured cells, as described herein.

We have used radioisotope based protocols to measure both HACU and ChAT enzyme activity in the same population of cultured cells. The protocols were originally developed to measure HACU in synaptosomes obtained from rodent brain (Simon et al., 1976) and ChAT enzyme activity in brain tissue (Fonnum, 1973). In our protocol, we have primarily used retinoic acid (RA) differentiated human neuroblastoma (SK-N-SH) cells. Human SK-N-SH cells display characteristics suggestive of multipotent embryonic precursor cells of neural crest origin (Pizzi et al., 2002). When exposed to RA, these cells undergo mitotic arrest and differentiate to a neuronal phenotype (Wainwright et al., 2001). Apart from differentiated SK-N-SH cells, we have also used this protocol to demonstrate HACU in embryonic rat primary cortical neuronal cells. Our simple, rapid and economical protocol not only accurately measures HACU in cell culture models, but at the same time assesses ChAT enzyme activity. Measurement of HACU and ChAT together in the same cells gives precise indication about the formation of acetylcholine and the "cholinergic status" of the cells. Taken together, this modified protocol can be used in cell culture-based drug development studies in many central nervous system disorders.

2. Results

2.1. HACU activity in differentiated and undifferentiated SK-N-SH cells

HACU was found to be present in both undifferentiated and differentiated SK-N-SH cells. However, as expected, all-*trans* retinoic acid (ATRA) differentiated SK-N-SH cells showed twice as much choline uptake compared to undifferentiated SK-N-SH cells under the same conditions of the assay (Fig. 1A).

2.2. ChAT enzyme activity in differentiated SK-N-SH cells

In addition to assaying HACU activity, enzymatic activity of ChAT was determined in differentiated SK-N-SH cells grown at two densities (100,000 and 200,000 cells/well). As shown in Fig. 1B, the differentiated SK-N-SH cells contained a significant ChAT activity, which was found to be approximately twice in 200,000 cells as in 100,000 cells, demonstrating linearity of enzyme activity with cell number.

2.3. Effect of incubation temperature on choline uptake

To optimize the incubation temperature for [^{3}H] choline uptake, we performed the uptake assay in differentiated SK-N-SH cells at four different temperatures, 7, 27, 37, and 47 °C. We observed a maximum choline uptake at 37 °C (Fig. 2A).

2.4. Effect of incubation time on choline uptake and ChAT enzyme activity

For HACU, we optimized the incubation time by incubating the differentiated SK-N-SH cells for 15, 30, 60, and 90 min. We observed that the choline uptake increased linearly up to 60 min of incubation and then reaches a plateau (Fig. 2B). We also conducted a time course for ChAT activity by incubating the cell lysate with ChAT buffer substrate (BS) for 15, 30, 45, and

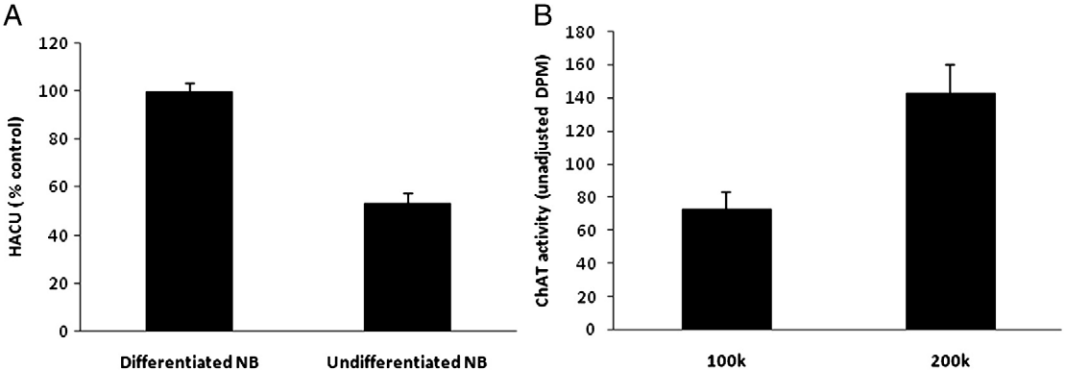

Fig. 1 – (A) High-affinity choline uptake in differentiated and undifferentiated SK-N-SH cells. Data for HACU were adjusted for CTG and are presented relative to differentiated cells SK-N-SH cells compared to the undifferentiated cells [*n*=3 for each group]. (B) Choline acetyltransferase activity as a function of cell number. ChAT activity was determined in differentiated SK-N-SH cells grown at two densities (100,000 and 200,000 cells/well). ChAT activity in wells that contain 200,000 cells is approximately twice that in wells containing 100 k cells, showing linearity of enzyme activity with cell number [*n*=3 for each group].