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Hemicholinium-3 sensitive choline transport in human T lymphocytes: Evidence for use as a proxy for brain choline transporter (CHT) capacity

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ABSTRACT

The synaptic uptake of choline via the high-affinity, hemicholinium-3-dependent choline transporter (CHT) strongly influences the capacity of cholinergic neurons to sustain acetylcholine (ACh) synthesis and release. To advance research on the impact of CHT capacity in humans, we established the presence of the neuronal CHT protein in human T lymphocytes. Next, we demonstrated CHT-mediated choline transport in human T cells. To address the validity of T cell-based choline uptake as a proxy for brain CHT capacity, we isolated T cells from the spleen, and synaptosomes from cortex and striatum, of wild type and CHT-overexpressing mice (CHT-OXP). Choline uptake capacity in T cells from CHT-OXP mice was two-fold higher than in wild type mice, mirroring the impact of CHT over-expression on synaptosomal CHT-mediated choline uptake. Monitoring T lymphocyte CHT protein and activity may be useful for estimating human CNS cholinergic capacity and for testing hypotheses concerning the contribution of CHT and, more generally, ACh signaling in cognition, neuroinflammation and disease.

1. Introduction

The cholinergic system of the brain is principally comprised of brainstem projections to midbrain, thalamic and striatal regions, basal forebrain projections to the cortex and other telencephalon regions, and local projections of striatal interneurons (Zaborszky et al., 2012). Among these cholinergic systems, the functions of the basal forebrain projection system are relatively well understood. Phasic and tonic cholinergic activity interacts in the cortex to integrate behaviorally relevant cues into ongoing behavior and to mediate attentional performance under taxing conditions (Sarter et al., 2014, 2016a; Gritton et al., 2016).

The capacity of cholinergic neurons to maintain elevated levels of neurotransmission is critically influenced by the neuronal choline transporter (CHT, *SLC5A7*). The capacity of the CHT to transport choline into the synapse is regulated tightly by mechanisms that determine the density and functionality of the CHT in the synaptosomal plasma membrane (Ferguson and Blakely, 2004; Ribeiro et al., 2006; Sarter and Parikh, 2005; Ennis and Blakely, 2016). Evidence supports the hypothesis that genetically-imposed, or spontaneously arising, variations in the capacity of the CHT impact levels of cholinergic neuromodulation and thereby modulate a range of fundamental cognitive, behavioral and physiological functions (Barwick et al., 2012; Ferguson et al., 2003, 2004; Holmstrand et al., 2014). For example, we previously demonstrated the cholinergic-attentional limitations that result from CHT heterozygosity in mice (Paolone et al., 2013; Parikh et al., 2013). In humans, a CHT single nucleotide polymorphism (I89V SNP; rs1013940) that bestows attenuated CHT capacity has been associated with high levels of attentional distractibility and distractor resistance as well as a bias for performing attention tasks using stimulus-driven (or bottom-up) strategies (Berry et al., 2014, 2015).

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This SNP has also been associated with attention deficit hyperactivity disorder and the severity of the symptoms of depression (English et al., 2009; Hahn et al., 2008). We also reported that a different CHT SNP (rs333229) may increase CHT capacity and is associated with distractor resistance and a bias for goal-directed attentional performance (Berry et al., 2014, 2015; Sarter et al., 2016b).

The impact of CHT SNPs or CHT mutations on CHT-mediated choline transport have been studied by expressing the cDNA encoding these variants in cell lines (Barwick et al., 2012; Okuda et al., 2002). However, currently there is no method to assess directly CHT function in humans. The presence of various cholinergic markers, including choline acetyltransferase (ChAT), acetylcholinesterase (AChE), the vesicular ACh transporter (VAChT) and muscarinic receptors in human T lymphocytes (Fujii et al., 1995, 1996; Tayebati et al., 2002) suggested to us that these readily accessible cells likely also express the CHT, to support their cholinergic signaling in the regulation of immune responses (Kawashima and Fujii, 2004; Rosas-Ballina et al., 2011). Indeed, Fujii and colleagues, in a short report, described the presence of the mRNA of the CHT in a human leukemic T cell line and that these cells exhibit choline uptake activity (Fujii et al., 2003). The goals of the present experiments were to determine the presence of the CHT protein in human T cells and to explore CHT-mediated, hemicholinium-3 (HC-3)-dependent choline transport, and to determine whether a previously studied genetically imposed variation of synaptosomal CHT capacity in mice (Holmstrand et al., 2014) can also be detected by measuring T cell based choline transport. The results collectively support the feasibility and potential significance of measuring human T cell choline uptake as a proxy for synaptosomal CHT capacity.

2. Methods

2.1. Subjects

CHT-OXP transgenic mice were originally generated at the Vanderbilt University Medical Center ES/Transgenic Core facility and maintained on an isogenic C57BL/6J genetic background as described earlier (Holmstrand et al., 2014). Male CHT-OXP breeder mice were shipped to the University of Michigan and backcrossed, for >9 generations, to obtain wild-type (WT) and CHT-OXP transgenic mutants. Animals were housed in a temperature-controlled (23 °C) and humidity-controlled (45%) environment with a 12 h light/dark cycle (lights on at 7:00 a.m.) with *ad libitum* food and water. Adult mice of either sex weighing 20–25 g at the beginning of the experiments were used. All procedures were conducted in adherence with protocols approved by the IACUC of the University of Michigan and in laboratories accredited by the Association for Assessment and Accreditation of Laboratory Animal Care.

2.2. Synaptosomal choline uptake

WT and CHT-OXP mice were decapitated under urethane anesthesia, brains were removed and tissues dissected on an ice-cold Petri dish. Right frontal cortices and striatal tissue from two mice were pooled to obtain sufficient amounts of tissue for the assay. Synaptosomal choline transport assays were performed as described in our previous publications (Holmstrand et al., 2014; Parikh et al., 2006, 2013; Parikh and Sarter, 2006). Briefly, aliquots (50 μL) of crude synaptosomes were incubated with 100 μL [^{3}H]-methyl choline chloride (0.02–6.0 μM, Perkin Elmer) in Kreb's bicarbonate buffer (118 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, 1.7 mM $CaCl_2$, 10 mM glucose, 100 μM ascorbic acid, and 10 μM physostigmine) in the presence and absence of 10 μM hemicholinium-3 (HC-3), a potent and selective CHT blocker (Guyenet et al., 1973), for 5 min at 37 °C. Transport assays were terminated by transferring the tubes to an ice bath followed by rapid filtration over a Brandel cell harvester (Brandel Inc. Gaithersburg, MD). Accumulated radioactivity was determined by scintillation spectrometry. Protein concentrations were measured using the Pierce BCA protein assay kit (Thermo Fisher Scientific Inc. Rockford, IL).

2.3. Human peripheral blood mononuclear cells (PBMCs) and T cell collection and purification

Completely de-identified plasmaphersis filters were obtained from the American Red Cross-Detroit Chapter in collaboration with Dr. Terry Smith (University of Michigan). Demographic information of these healthy donors was not available due to de-identification process. However, these subjects were adults that have been considered as healthy to donate plasma for medical uses. PBMC were removed from plasmapheresis filters by washing in reverse flow with 30 mL of phosphate-buffered saline (PBS), overlaid onto 15 mL of Ficoll-Paque™ (GE healthcare Bio-Sciences AB, Uppsala, Sweden) in a 50-mL conical centrifuge tube, followed by centrifugation at 400 × g for 40 min at room temperature without brake. Mononuclear cells were collected from the interphase between Ficoll and upper aqueous buffer phase, washed twice with PBS. T cells were then purified using human Pan-T isolation kit from Miltenyi Biotec Inc (San Diego, CA, USA) according to manufacturer recommended procedure. In brief, non-T cells were bound with biotin conjugated antibodies against CD14, CD16, CD19, CD36, CD56, CD123, and glycophorin A. These cells were captured with anti-biotin microbeads and magnetically depleted via column pass-through (negative selection) to produce a highly pure (90–97%) population of untouched pan T cells.

2.4. Flow cytometry analysis of PBMCs

Human leukocytes from unidentified healthy donors were back-flushed from ATS™ LPL Filters (Pall Medical, East Hills, NY, USA), obtained from blood bank, using 30 mL of phosphate-buffered saline (PBS). The leukocytes mix were overlayed onto 15 mL of Ficoll-Paque Plus (GE Healthcare Life Sciences, Pittsburgh, PA, USA), followed by centrifugation at 400 × g for 40 min. PBMCs were carefully collected from the interphase of upper and lower phases, washed and counted. 1×10^6 PBMCs were surface stained with FITC-conjugated anti-$TCR_{\alpha\beta}$, PE-conjugated anti-CD4 (BioLegend, San Diego, CA), 7-amino-actinomycin D, PE-Cy7-conjugated anti-CD19 (Tonbo Biosciences, San Diego, CA), APC-Cy7-conjugated anti-CD14 (BD Biosciences, East Rutherford, NJ), APC-conjugated anti-CD8 (Tonbo Biosciences, San Diego, CA) and Brilliant Violet 510-conjugated anti-$TCR_{\gamma\delta}$ (BioLegend, San Diego, CA), as well as unconjugated anti-choline transporter antibody (SLC5A7, extracellular) from Alomone Labs (Jerusalem, Israel). After washing to remove unconjugated antibodies, cells were stained with or without Brilliant Violet 421-conjugated Donkey anti-rabbit IgG. The cells were then washed again with PBS containing 2% of heat-inactivated fetal bovine serum and analyzed on a BD FACS CantoII flow cytometer (BD Biosciences, San Jose, CA, USA).

2.5. T cell collection in mice

WT and CHT-OXP mice were sacrificed and single cell