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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.

Keywords

choline transporter; T lymphocytes; synaptosomes; acetylcholine; genetic CHT variants

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Author Contributions. A.K.C., R.D.B. and M.S. designed the research and analyzed the data. A.K.C. analyzed the data. V.P. conducted immunoblotting experiments. Y.M.D., Qi W. and Qin W. isolated human PBMCs and T cells. A.K.C. conducted choline uptake assays. All authors contributed to the writing of the paper.

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). 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-OMP 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-OMP breeder mice were shipped to the University of Michigan and backcrossed, for >9 generations, to obtain wild-type (WT) and CHT-OMP 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-OMP 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 [³H]-methyl choline chloride (0.02–6.0 µM, Perkin Elmer) in Krebs' bicarbonate buffer (118 mM NaCl, 4.7 mM KCl, 1.2 mM KH₂PO₄, 25 mM NaHCO₃, 1.7 mM CaCl₂, 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 plasmapheresis 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 ATSTM LPL Filters (Pall Medical, East Hills, NY, USA), obtained from blood bank, using 30 mL of phosphate-buffered saline (PBS). The leukocytes mix were overlaid onto 15 mL of Ficoll-Paque Plus (GE Healthcare Life Sciences, Pittsburgh, PA, USA), followed by centrifugation at $400 \times g$ 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-OMP mice were sacrificed and single cell suspensions were prepared from spleens. Briefly, the tissue was homogenized in $1 \times$ PBS buffer and filtered through Falcon 40- μ m cell strainer (Corning, NY) in to 50 mL conical tubes. The suspension was centrifuged at $530 \times g$ for 4 min at 4° C. The supernatant was discarded and the pellet re-suspended in 1.66% ammonium chloride solution for erythrocyte lysis. The cells were washed twice and the pellet was re-suspended in $1 \times$ PBS buffer. Cells were then counted and T cells were isolated by positive selection using anti CD90.2 microbeads (Roggia et al., 2001) and the MACS magnetic system (MACS Miltenyi Biotec, Auburn, CA). T cells were again counted and centrifuged at $300 \times g$ for 10 min at 4° C to collect the pellet.

2.6. Choline uptake assay in T cells

T cell pellets were diluted in assay buffer to yield 10^6 cells per 50 μ L sample. Aliquots of 50 μ L sample were incubated with two concentrations of 100 μ L [3H]-methyl choline chloride (0.002 μ M and 2 μ M for mouse T cells; 0.006 μ M and 6 μ M for human T cells) in the presence and absence of HC-3 (10 μ M) for 20 min at 37° C. Subsequent procedures were similar to those used for determining synaptosomal choline uptake described above.

2.7. Immunoblotting

T cell pellets were lysed in 250 μ L of 50 mM HEPES NaOH homogenization buffer (pH 7.4–7.6) containing 0.3M sucrose, 0.25 mM $MgCl_2$, 1mM EDTA, 1mM EGTA, and protease inhibitor cocktail (1.0 μ g/mL leupeptin, 1.0 μ g/mL aprotinin, 1.0 μ g/mL pepstatin, and 250 μ g/mL phenylmethylsulfonyl fluoride). The homogenate was centrifuged at $10,000 \times g$ for 10 min and the supernatant was collected and stored at $-80^\circ C$ until further processing. Protein concentrations in lymphocyte lysates were determined by using a modified Lowry Protein Assay (Pierce). CHT immunoblotting procedure was adapted from previous studies (Apparsundaram et al., 2005; Ferguson et al., 2003; Parikh and Sarter, 2006; Parikh et al., 2013). Briefly, lysates containing equal quantities (25 μ g) of protein were loaded on the 4–15% Tris HCl polyacrylamide gel and each sample was assayed in duplicate. Proteins were separated using electrophoresis and were transferred to the PVDF membrane. A rabbit anti-CHT polyclonal antibody (ABN458; Millipore) was used at 1:2000 dilution for the immunodetection of CHT bands. The membrane was incubated with a peroxidase-conjugated anti-rabbit secondary antibody and ECL Advance chemiluminescent substrate (GE Healthcare) for the detection of chemiluminescent signal using the Molecular Imager Chemidoc EQ system (Bio-Rad). β -actin served as a gel loading control and was detected using the mouse monoclonal antibody (Santa Cruz; 1:1000 dilution). Densitometric analysis of CHT-immunoreactive bands was performed by calculating the integrated pixel densities using NIH ImageJ software. The density of CHT-immunoreactive bands was normalized to the levels of β -actin-immunoreactive bands for each sample analyzed.

2.8. Statistical analyses

Statistical analyses were performed using the SPSS for Windows (version 23.0: SPSS). For ANOVAs, assumptions underlying the statistical model were assessed. In cases of violation of the sphericity assumption, Huynh–Feldt-corrected F values, along with uncorrected degrees of freedom, are given. Alpha was set at 0.05. Exact P values are reported as recommended previously (Greenwald et al., 1996).

3. Results

3.1. Presence of CHT and CHT-mediated choline transport in human T cells

T cells were previously shown to be immunoreactive for ChAT, AChE and VACHT, and to produce ACh (Fujii et al., 1995; Rosas-Ballina et al., 2011; Tayebati et al., 2002). Thus we hypothesized that these cells also express the neuronal CHT. T cells were isolated from human blood and processed for immunoblotting as detailed in Methods. Choline transport was determined in the absence and presence of the CHT blocker hemicholinium-3 (HC-3; 10 μ M). Immunoblotting indicated a robust presence of the CHT in human T cells (Fig. 1a). Furthermore, choline transport was present and highly dependent on the availability of the CHT (10^6 T cells; Fig. 1b). Indeed, HC-3-induced CHT blockade reduced T cell choline uptake by >90%, and therefore robustly more so than observed in rodent synaptosomes (below). As HC-3-independent choline transport is generally assumed to reflect uptake via low-affinity sites that, in neurons, support phospholipid synthesis (Michel et al., 2006), this finding suggests a near absence of low-affinity sites in T cells. Reflecting the near absence of non-specific choline transport, choline uptake in the presence of a relatively low (0.066

μM) or relatively high ($6 \mu\text{M}$) choline concentrations yielded similar levels of choline uptake (Mann-Whitney $U=7$, $P=0.09$), indicating that the high-affinity transporter dominates choline uptake in human T cells.

To generate a range of potential V_{max} values for T cell choline uptake, we constructed a series of saturation curves taken as a function of choline concentrations lower than $0.066 \mu\text{M}$. The resulting V_{max} values ranged between, 0.03 and $0.12 \text{ pmol/mg protein/min}$, consistent with a ~ 40 -fold lower choline transport capacity of T cells as compared with mouse synaptosomes ($2\text{--}4 \text{ pmol/mg/protein/min}$) (Holmstrand et al., 2014).

3.2. CHT expression in subpopulations of human T cells

The presence of enzymes associated with the synthesis and release of ACh may be restricted to certain subpopulations of human T cells (Fujii et al., 2003). We therefore employed flow cytometry analysis to determine the expression of the CHT in T cell subpopulations. Peripheral blood mononuclear cells (PBMCs) purified from healthy donors were stained with fluorochrome-conjugated antibodies against B lymphocytes, monocytes, T cell $\alpha\beta$ and $\gamma\delta$ receptors, CD4 and CD8, to identify specific peripheral T cell populations (Fischer et al., 2005; Pinheiro et al., 2012). A polyclonal rabbit anti-extracellular CHT (SLC5A7) followed by fluorochrome-conjugated donkey anti-rabbit IgG were used to identify cells expressing the CHT (see Methods).

As illustrated in Fig. 2b, $25.8 \pm 3.0\%$ of $\text{TCR}_{\alpha\beta}^+$ T cells and $46.4 \pm 17.7\%$ of $\text{TCR}_{\gamma\delta}^+$ T cells were positive for the CHT. Among $\text{TCR}_{\alpha\beta}^+$ T cells, $24.1 \pm 3.2\%$ of CD4^+ helper T cells and $27.7 \pm 3.5\%$ CD8^+ cytotoxic T cells expressed the CHT. CD4^+ and CD8^+ T cells were further classified into naïve T cells (Tn; $\text{CD45RA}^+/\text{CD45RO}^-/\text{CCR7}^+$), antigen experienced central memory T cells (Tcm; $\text{CD45RA}^-/\text{CD45RO}^+/\text{CCR7}^+$), effector memory T cells (Tem; $\text{CD45RA}^-/\text{CD45RO}^+/\text{CCR7}^-$) and effector memory RA T cells (Temra; $\text{CD45RA}^+/\text{CD45RO}^-/\text{CCR7}^-$; Fig. 2c). Among CD4^+ T cells, $31.4 \pm 6.7\%$ of Tn, $24.1 \pm 7.5\%$ of Tcm, $24.5 \pm 8.2\%$ of Tem and $33.0 \pm 13.4\%$ of Temra cells expressed the CHT. Among CD8^+ T cells, $30.0 \pm 10.8\%$ of Tn, $46.1 \pm 13.9\%$ of Tcm, $33.9 \pm 13.5\%$ of Tem and $36.7 \pm 15.0\%$ of Temra expressed CHT (Fig. 2e,f). Overall, approximately 25–30% of T cells of all different subsets analyzed expressed the CHT.

3.3 Genetically-imposed elevation of CHT-mediated choline transport in mouse synaptosomes and T cells

In the absence of human synaptosomes for validating T cell choline uptake as an index of neuronal CHT capacity, we determined whether a genetically imposed CHT capacity variation in mice yields comparable alterations on mouse synaptosomal and mouse T cell choline uptake. Previously, we reported mice expressing CHT from a multi-copy insertion of a bacterial artificial chromosome (BAC)-insertion of the *Slc5a7* gene (Holmstrand et al., 2014). Immunohistochemistry revealed markedly elevated CHT expression in the cell bodies of cholinergic neurons and axons projecting to regions known to receive cholinergic innervation. Biochemical studies indicated a 2- to 3-fold elevation in total CHT protein levels in the CNS and comparable elevations in CHT activity (Holmstrand et al., 2014). CHT

densities are also markedly higher in both synaptic plasma membrane and intracellular fractions, paralleled by significant increases in [^3H]HC-3 binding (Holmstrand et al., 2014).

Synaptosomal, HC-3-sensitive choline uptake in CHT-OMP reached about twice the levels of uptake seen in WT mice, irrespective of brain region (effects of genotype on choline uptake without HC-3; cortex: $F(1,7)=8.68$, $P=0.02$; WT: 1.47 ± 0.39 pmol/mg/min (M, SEM); CHT-OMP: 3.20 ± 0.44 ; effects of choline concentration: $F(5,35)=73.65$, $P<0.001$; genotype $\times$ concentration: $F(5,35)=9.13$, $P=0.008$; Fig. 2a; striatum: $F(1,7)=20.20$, $p=0.03$; WT: 2.13 ± 0.33 pmol/mg/min (M, SEM); CHT-OMP: 4.37 ± 0.37 ; effects of choline concentration: $F(5,35)=75.22$, $P<0.001$; genotype $\times$ concentration: $F(5,35)=9.81$, $P=0.003$; Fig. 2b). These results are consistent with our previously reported V_{max} values in WT and CHT-OMP mice (no difference in K_{M} (Holmstrand et al., 2014)). Choline uptake in T cells (10^6 T cells) obtained from WT mice matched choline transport determined in human T cells, with maximum uptake of approximately 0.1 pmol/mg protein/min (Fig. 1e). In T cells obtained from CHT-OMP mice, choline uptake doubled in the presence of the higher concentration of choline (effects of genotype on choline uptake without HC-3: $F(1,4)=14.12$, $P=0.02$; effects of choline concentration and genotype $\times$ concentration: both $P>0.10$). Thus, the increase in T cell choline uptake corresponded with that observed in brain synaptosomes (Fig. 1e). The CHT-OMP-associated near doubling of CHT protein concentrations in T cells (normalized integrated CHT density (arbitrary units): WT: 78.36 ± 3.78 ; CHT-OMP: 107.54 ± 5.45 ; Fig. 1f) corresponded with the increase in choline transport measured in T cells from CHT-OMP mice.

4. Discussion

The results from the present experiments indicate that the neuronal CHT is present in human and mouse T lymphocytes and that these cells transport choline via the CHT. Furthermore, we demonstrated that the functional impact of a genetically imposed increase in synaptosomal plasma membrane CHT density generalizes to T cells obtained from these mice. Together, these results support the potential use of T lymphocyte-based choline transport as a proxy measure for indicating brain synaptosomal CHT capacity. The finding that choline transport in 10^6 T cells from human blood and mouse spleen was nearly identical confirms the feasibility and reliability of this assay.

In T lymphocytes, CHT density and the capacity for CHT-mediated uptake were robustly lower than in synaptosomes, perhaps by a factor of 40. However, comparisons between results from synaptosomal and T cell choline uptake assays require extreme caution, primarily because of heterogeneous amounts and sources of protein. Indeed, T cell choline uptake may preferably be expressed as a function of the number of cells per assay, to prevent direct and inconclusive comparisons based on protein concentration and thus with results on synaptosomal uptake.

4.1. Limitations of the current evidence

Several limitations apply to the present results. First, though HC-3 sensitivity and the CHT gene-dosage dependence on choline uptake supports CHT as the source of choline uptake in T cells, the near saturation of choline uptake at relatively low nanomolar levels of choline is

surprising given the micromolar K_M observed in brain preparations (Yamamura and Snyder, 1972; Holmstrand et al., 2014). These findings may indicate distinct demands for choline uptake/clearance in the extracellular milieu of synaptosomes and T cells, including differences between interacting proteins and post-translational mechanisms that alter CHT conformation. Further mechanistic studies, including full saturation analysis, examination of effects of lower HC-3 concentrations, and sodium-dependency studies (Simon et al., 1976) are also warranted.

Furthermore, it is presently not clear whether T cells contain an intracellular pool of CHT and employ intracellular CHT trafficking steps that correspond with those observed in synapses (Ribeiro et al., 2006, 2007). Therefore, the effects of CHT heterozygosity, or of variations in the mechanisms that control CHT mobilization and the status of CHT in synaptosomal plasma membrane (Gates et al., 2004), may not necessarily be reproduced in T cells. However, as T cells release ACh (Rinner et al., 1998), associated CHT mobilization may mirror neuronal CHT intracellular trafficking mechanisms more closely than currently speculated.

4.2. Proxy for synaptosomal uptake

T cell-based CHT-mediated choline transport as a proxy for brain synaptosomal choline transport may be most useful for identifying CHT capacity variances in non-genotyped populations as well as in assessing the impact of defined CHT variants and mutations (references in Introduction). The present demonstration of parallel effects of CHT overexpression on synaptosomal and T cell choline transport is only a first step toward validation of this method. The future demonstration that T cells from humans heterozygous or homozygous for the CHT subcapacity I89V SNP (Okuda et al., 2002) both exhibit attenuated CHT function will represent an important step indicating the usefulness of this method.

We do not exclude that other peripheral blood cells also express the CHT and exhibit CHT-mediated choline transport (see, for example, the presence of the serotonin transporter in platelets; Beikmann et al., 2013). However, it is noteworthy that a stable proportion (about 30%) of T cells of all subclasses expressed the CHT.

4.3. Potential usefulness of measures of T cell CHT capacity

As described in the Introduction, the capacity of the CHT limits levels of cholinergic neuromodulation and humans expressing genetic CHT variants exhibit attentional biases that are associated with low versus high levels of cholinergic neuromodulation (Sarter et al., 2016b). Future studies are expected to show relationships across genotypes, and potentially within genotypes, between levels of CHT capacity and levels of attentional control. Furthermore, as CHT subcapacity variants are hypothesized to be associated with elevated risks for neuropsychiatric and neurological disorders (English et al., 2009; Hahn et al., 2008; Koshy Cherian et al., 2015), measures of T cell CHT capacity offers a proxy neuromarker for testing this hypothesis.

ACh-releasing T cells are an integral part of the inflammatory reflex. Stimulation of T cells and resulting ACh release dampens cytokine production and thus improves the course of

inflammatory processes (Rosas-Ballina et al., 2011). The present results indicate that T cells express the neuronal CHT and that therefore these cells contain the full set of proteins involved in neuronal ACh synthesis. Furthermore, if it is the case that CHT subcapacity variants, such as the I89V SNP, attenuates the capacity of T cells to transport choline and thus to synthesize and release ACh, it would be intriguing to determine whether such variants are associated with relatively weak immunological responses to inflammatory challenges or a heightened propensity for neuroinflammatory events. Furthermore, and given that depression is increasingly associated with abnormal pro-inflammatory processes (Wohleb et al., 2016), such a finding would begin to explain evidence indicating an association between the CHT subcapacity I89V variant and depression severity (Hahn et al., 2008). In conclusion, T cell-based assessment of CHT function may serve as an important translational tool for studying the role of cholinergic systems in health and disease.

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Highlights

- The neuronal choline transporter is present in human and mouse peripheral T cells.
- Choline uptake by T cells is nearly completely blocked by hemicholinium-3.
- CHT overexpression increases choline uptake in synaptosomes and T cells.
- Choline uptake by T cells serves as a proxy for brain choline transporter function.

Figure 1.

Human T cells express the neuronal CHT and transport choline in a HC-3 dependent manner (a,b) and comparable increases in CHT-mediated choline transport by brain synaptosomes and T cells from mice over-expressing the CHT (CHT-OXP; c–f). **a:** Western blots show the presence of the neuronal CHT in human T lymphocytes (normalized against actin; each lane is based on T cells from one individual; 25 μg protein loaded in each well). **b:** Choline uptake in human T cells (10^6 cells) in the absence and presence of hemicholinium-3 (HC-3; data are based on 6 samples/6 humans). Comparable increases in CHT-mediated choline transport by brain synaptosomes and T cells from mice over-expressing the CHT (CHT-OXP). Choline uptake by synaptosomes obtained from frontal cortex (c) or striatum (d) of wildtype (WT) and CHT-OXP mice indicates an approximate doubling of choline uptake in CHT-OXP (as is also indicated in our prior report of V_{max} values in Holmstrand et al., 2014). **e:** Corresponding with the effects of CHT over-expression in synaptosomes, T cells (10^6 cells) obtained from spleens of CHT-OXP mice had about twice the capacity for choline uptake than spleen-derived T cells from WT mice. **f:** immunoblotting indicated the presence of the CHT in T cells collected from the spleen of mice and, corresponding with the elevated expression of the CHT in synaptosomes from CHT-OXP mice (Holmstrand et al., 2014), elevated CHT expression in T cells obtained from CHT-OXP (the two lanes depict lysate samples in duplicates per genotype (25 μg protein loaded in each well).

Figure 2.

CHT expression in T cell subpopulations. PBMCs from 8 healthy donors were surface stained with various fluorochrome conjugated antibodies and analyzed using flow cytometer to identify the proportion of specific T cell subpopulations expressing the CHT. **a:** Representative CHT staining profile of one subject's total TCR $\alpha\beta$ ⁺ T cells, CD4⁺CD8⁻ T cells, CD4⁻CD8⁺ T cells, and TCR $\gamma\delta$ ⁺ T cells. Darker lines represent the expression profile when cells were stained with anti-CHT and lighter lines represent the profile in the absence of anti-CHT (control). **b:** CHT expression profile of the samples from 8 healthy donors

(medians and 95% confidence intervals). **c**: Schematic illustration of the T cell differentiation subsets using the markers CCR7 and CD45RO (Tn, naïve T cells; Tcm, central memory T cells; Tem, effector memory T cells; Temra, terminally differentiated effector T cells). **d**: CD4⁺CD8⁻ and CD4⁻CD8⁺ T cells were further differentiated using the markers CCR7 and CD45RO as depicted in (c). **d** shows representative CHT staining profiles of the different subsets of CD4⁺CD8⁻ and CD4⁻CD8⁺ from one human subject. CHT expression profiles in Tn, Tcm, Tem, and Temra cells that are also CD4⁺CD8⁻ T cells (**e**) or CD4⁻CD8⁺ T cells (**f**) from all 8 donors (medians and 95% confidence intervals).