Normalization of Cholesterol Homeostasis by 2-Hydroxypropyl-β-cyclodextrin in Neurons and Glia from Niemann-Pick C1 (NPC1)-deficient Mice*#S

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_**Background:**_ Cyclodextrin (CD) mobilizes stored cholesterol and delays neurodegeneration in Niemann-Pick C (NPC) mice.

_**Results:**_ 0.1 mM CD increased, whereas 1 mM decreased, ER cholesterol; 10 mM CD was neurotoxic.

_**Conclusion:**_ CD liberated cholesterol from lysosomes of NPC1-deficient neurons and glia and modulated cholesterol homeostasis.

_**Significance:**_ A therapeutic dose of CD for NPC patients is indicated.

Niemann-Pick C (NPC) disease is an inherited, progressive neurodegenerative disorder caused by mutations in the NPC1 or NPC2 gene that result in an accumulation of unesterified cholesterol in late endosomes/lysosomes (LE/L) and impaired export of cholesterol from LE/L to the endoplasmic reticulum (ER). Recent studies demonstrate that administration of cyclodextrin (CD) to Npc1<sup>−/−</sup> mice eliminates cholesterol sequestration in LE/L of many tissues, including the brain, delays neurodegeneration, and increases lifespan of the mice. We have now investigated cholesterol homeostasis in NPC1-deficient cells of the brain in response to CD. Primary cultures of neurons and glial cells from Npc1<sup>−/−</sup> mice were incubated for 24 h with 0.1 to 10 mM CD after which survival and cholesterol homeostasis were monitored. Although 10 mM CD was profoundly neurotoxic, and altered astrocyte morphology, 0.1 and 1 mM CD were not toxic but effectively mobilized stored cholesterol from the LE/L as indicated by filipin staining. However, 0.1 and 1 mM CD altered cholesterol homeostasis in opposite directions. The data suggest that 0.1 mM CD releases cholesterol trapped in LE/L of neurons and astrocytes and increases cholesterol availability at the ER, whereas 1 mM CD primarily extracts cholesterol from the plasma membrane and reduces ER cholesterol. These studies in Npc1<sup>−/−</sup> neurons and astrocytes establish a dose of CD (0.1 mM) that would likely be beneficial in NPC disease. The findings are timely because treatment of NPC disease patients with CD is currently being initiated.

Dysregulation of cholesterol metabolism in the brain has been implicated in neurodegenerative disorders such as Alzheimer disease, Huntington disease, Smith-Lemli-Opitz syndrome, and Niemann-Pick type C (NPC) disease. NPC disease is a fatal, progressive, autosomal recessive disorder caused by mutations in the NPC1 or NPC2 gene (1, 2). The symptoms of NPC disease, including progressive neurodegeneration as well as liver and lung disease, typically begin in childhood and cause premature death (reviewed in Ref. 3). The NPC1 and NPC2 proteins act sequentially in releasing cholesterol derived from endocytosed LDL from late endosomes/lysosomes (LE/L) (4). Thus, in NPC1- or NPC2-deficient cells unesterified cholesterol and other lipids are sequestered in LE/L so that cholesterol transport from LE/L to the plasma membrane (5) and endoplasmic reticulum (ER) (6) is attenuated. The ER cholesterol concentration dictates the processing of sterol response element-binding protein-2 (SREBP2), which transcriptionally regulates the expression of many genes involved in cellular cholesterol homeostasis (7). Thus, when the ER cholesterol content exceeds ~5% of total lipids (6), the expression of genes involved in cholesterol accretion is reduced whereas cholesterol storage as cholesteryl esters (CE), and expression of genes involved in cholesterol efflux, are increased. Consequently, cholesterol homeostasis is impaired in NPC1-deficient cells.

A treatment for NPC disease has been elusive. However, Liu _et al._ (8) reported recently that a single injection of 2-hydroxypropyl-β-cyclodextrin (CD) into 7-day-old Npc1<sup>−/−</sup> mice delayed neurodegeneration and significantly prolonged survival. Moreover, serial injections of CD further increased lifespan of Npc1<sup>−/−</sup> as well as Npc2<sup>−/−</sup> mice (9, 10). The CD treatments reduced cholesterol accumulation in LE/L, increased the amount of CE, and decreased cholesterol synthesis _in vivo_ in livers and brains of Npc1<sup>−/−</sup> mice (8). CD also decreased the amounts of mRNAs encoding SREBP2 and its target genes and

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*This work was supported by the Ara Parseghian Medical Research Foundation and Canadian Institutes for Health Research (to J. E. V.) as well as graduate studentships from the Natural Sciences and Engineering Research Council of Canada and the Alberta Heritage Foundation for Medical Research (to K. B. P.).

□ This article contains supplemental Table S1.

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2 The abbreviations used are: NPC, Niemann-Pick type C; ABC, ATP-binding cassette; protein; ACAT, acyl-CoA:cholesterol acyltransferase; CD, 2-hydroxypropyl-β-cyclodextrin; CE, cholesteryl esters; HMGCR, 3-hydroxy-3-methylglutaryl-CoA reductase; LE/L, late endosomes/lysosomes; LDLR, LDL receptor; SREBP, sterol-response element-binding protein; qPCR, quantitative PCR.
increased mRNAs involved in cholesterol efflux in several tissues (8, 9). CD binds cholesterol with high affinity (11), and a high dose of CD (5–10 mM for 10 min) is often used experimentally to extract cholesterol from the plasma membrane of cells but can cause cell death (12). Lower doses of CD, however, appear to enter cells by bulk-phase endocytosis and bypass functions of NPC1/NPC2 so that sequestered cholesterol is mobilized from the LE/L (13). Although CD crosses the blood-brain barrier of mice inefficiently, some (~0.2%) plasma CD does enter the brain (14).

These findings indicate that CD might be useful for treatment of NPC patients. CD is relatively non-toxic and is used in humans for drug delivery (12). In 2010, the FDA approved peripheral injection of CD for “compassionate use” in NPC patients and direct delivery of CD to the brain is being considered currently. Nevertheless, many questions remain concerning the mechanism by which CD acts in the brain, the organ most profoundly affected in NPC disease. Because previous studies with CD were performed in intact Npc1−/− mice (8, 9, 14), the types of brain cells affected by CD were unknown. Thus, we have established the dose of CD that is tolerated by neurons and glial cells from Npc1−/− mice and have investigated mechanisms by which CD produces beneficial effects on cholesterol homeostasis in these cells.

**EXPERIMENTAL PROCEDURES**

**Materials**—2-Hydroxypropyl-β-cyclodextrin (H107), poly-D-lysine hydrobromide, trypsin (type XII-S, bovine pancreas), and filipin were from Sigma. DMEM, Ham’s F12 medium, Neurobasal™ medium, FBS, B-27 supplement, 0.25% trypsin-EDTA, Hoechst 33258 pentahydrate, DNase I (amplification grade), oligo(dT)12–18 primer, SuperScript® II, Platinum® qPCR SuperMix-UDG, and SYBR Green I were from Invitrogen. Culture flasks (75-cm²), 96-well plates, and 100 × 20 mm culture dishes were from BD Falcon (BD Biosciences), and 60 × 15 mm culture dishes were from Corning, Inc. (Corning, NY). [1-14C]Oleic acid was from PerkinElmer Life Sciences, and sodium [3H]acetate and CytoScint™ were from MP Biomedicals (Solon, OH). PCR-grade dNTPs (deoxynucleoside triphosphates) were from Roche Applied Sciences.

**Npc1+/+ and Npc1−/− Mice**—A breeding colony of Balb/cNctr-Npc1+/+ mice from Jackson Laboratories (Bar Harbor, ME) was maintained at the University of Alberta under temperature-controlled conditions with a 12-h light:12-h dark cycle. Npc1−/− mice were used for breeding. Mice were supplied with 9% fat breeders’ diet (Purina LabDiet, Richmond, IN) and water ad libitum. The Npc1 genotype was determined by PCR analysis of genomic DNA from tail clippings using REDExtract-N-Amp™ tissue PCR kit (Sigma) (23). Npc1+/+ littermates were used as controls. All experiments were approved by the University of Alberta Animal Welfare Committee.

**Culture of Cortical Astrocytes and Microglia**—Glial cells were isolated from cerebral cortices of 1- to 3-day-old Npc1+/+ and Npc1−/− mice (23, 24) and plated at a density of 2 cortices/75-cm² flask or one cortex/100 × 20 mm dish. Cells were maintained at 37 °C and 5% CO₂ in DMEM containing 10% FBS. The medium was replaced every 3–5 days. After 3–4 weeks, confluent glial cells were washed in phosphate-buffered saline, harvested with 0.125% trypsin and replated at a density of 1:3 in DMEM containing 10% FBS. The cultures were highly enriched (>90%) in astrocytes (24) and were used within 7–14 days. Microglia were isolated from confluent glial cultures by mild trypsinization (25).

**Radiolabeling of Cholesterol and CE**—Neurons and astrocytes were cultured for 7 days in 96-well plates and then washed and fixed for 15 min in 4% (w/v) paraformaldehyde. For assessment of cell death by apoptosis, cells were stained at room temperature for 12 min with 0.5 µg/ml Hoechst 33258. Stained nuclei were counted, and cells containing shrunken or fragmented nuclei were scored as apoptotic (24). The number of apoptotic cells was calculated as % of total number of neurons. For qualitative assessment of cholesterol distribution cells were stained for 1.5 h with 0.15 mg/ml filipin (23). Cells were examined with a Leica DM IRE2 fluorescence microscope with an excitation wavelength of 351 nm for Hoechst and filipin.

**Staining with Filipin and Hoechst Dye**—Neurons and astrocytes were cultured for 7 days in 60 × 15 mm dishes prior to CD treatment. For cholesterol labeling, cells were incubated with 5 µCi/ml [3H]acetate and 100 µM sodium acetate for 4 h. For cholesteryl esterification assays, astrocytes or neurons were incubated for 4 or 24 h, respectively, with 0.2 µCi/ml [14C]oleate, 100 µM oleic acid, 0.5% bovine serum albumin ± 2 µg/ml Sandoz 58-035 (Sigma). Lipids were extracted with chloroform/methanol (2:1), washed with methanol/water (1:1), and separated by thin-layer chromatography in the solvent system heptane/isopropanol ether/acetic acid/isopropanol (65:35:4:2). Bands corresponding to cholesterol, CE, and phospholipids were scraped, and radioactivity was quantified. Protein concentrations were determined using the BCA™ protein assay (Thermo Scientific, Rockford, IL).

**RNA Isolation and Real-time qPCR**—Neurons were cultured for 7 days in 75-cm² flasks, whereas astrocytes were cultured for 14 days in 100 × 20 mm dishes. Total RNA was isolated using the RNasy mini kit (Invitrogen) and stored at −80 °C. RNA was treated with DNase I, then cDNA was synthesized from 1.25 µg total RNA using oligo(dT)12–18 random primers and Superscript II reverse transcriptase according to the manufacturer’s instructions. qPCR reactions were performed with Platinum® Quantitation PCR supermix, SYBR Green I, and 250 nmol of gene-specific primers. qPCR analysis was performed on a Rotor-Gene 3000 instrument (Montreal Biotech, Montreal.
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FIGURE 1. Intracellular cholesterol sequestration in Npc1+/− neurons and glia. Cerebellar granule neurons (A and B), cortical astrocytes (C and D), and cortical microglia (E and F) from Npc1−/− and Npc1+/− mice were cultured for 7 days. The distribution of unesterified cholesterol was qualitatively assessed by filipin staining. Similar results were obtained in at least three additional experiments.

RESULTS

Cholesterol is sequestered in LE/L of NPC1-deficient cells because the export of LDL-derived cholesterol from LE/L is impaired (15). Qualitative comparison of filipin staining of unesterified cholesterol in primary cultures of cerebellar granule neurons (Fig. 1, A and B), cortical astrocytes (Fig. 1, C and D) and cortical microglia (Fig. 1, E and F) from Npc1+/− and Npc1−/− mice shows that NPC1 deficiency in all these cells induces an intracellular accumulation of unesterified cholesterol in a punctate distribution typical of that in other Npc1−/− cells. In Npc1+/− neurons, astrocytes, and microglia, filipin staining was much less pronounced than in Npc1−/− cells (Fig. 1, A–F).

Low Concentrations of CD Are Not Toxic and Reduce Cholesterol Sequestration in Npc1−/− Neurons and Astrocytes—High concentrations of CD (5–10 mM) typically deplete cells of cholesterol and can cause cell death (11, 12, 16). Nevertheless, CD is more toxic to some cell types than to others (reviewed in Ref. 16). To determine which CD concentrations are tolerated by neurons and astrocytes from Npc1−/− and Npc1+/− mice, we incubated the cells for 24 h with 0.1–10 mM CD. Phase-contrast microscopy revealed that morphology of Npc1+/+ (Fig. 2, A–C) and Npc1−/− (Fig. 3, A–C) neurons was unaltered by 0.1 or 1 mM CD. However, no Npc1+/+ (Fig. 2D) or Npc1−/− (Fig. 3D) neurons survived after a 24-h incubation with 10 mM CD. Treatment of Npc1+/+ (Fig. 2, E–G) or Npc1−/− (Fig. 3, E–G and J) astrocytes with 0.1 or 1 mM CD did not change cell morphology or induce cell death, whereas 10 mM CD markedly altered morphology of Npc1+/+ (Fig. 2H) and Npc1−/− (Fig. 3H) astrocytes, although the detrimental effect in astrocytes was not as profound as in neurons. The number of apoptotic nuclei in the cultured Npc1+/+ and Npc1−/− neurons and astrocytes was quantified by Hoechst staining. Neither 0.1 nor 1 mM CD decreased survival of Npc1+/+ (Fig. 2, I and J) or Npc1−/− (Fig. 3, I and J) neurons. Thus, exposure of neurons and astrocytes to 0.1 and 1 mM CD for 24 h does not alter cell morphology or compromise survival. We performed no further experiments with 10 mM CD because this concentration was neurotoxic.

To determine whether 0.1 and 1 mM CD released sequestered cholesterol from LE/L of Npc1−/− neurons, astrocytes, and microglia, the cells were incubated with CD for 24 h and then examined by filipin staining. The intracellular punctate filipin staining essentially was eliminated in all three types of Npc1−/− cells (Fig. 4). Weak filipin staining, primarily on the cell surface, was evident in Npc1+/+ neurons (Fig. 5, A–C) and astrocytes (Fig. 5, D–F) and was not significantly altered by CD. These data indicate that low doses (0.1 and 1 mM) of CD mobilized LE/L cholesterol in Npc1−/− neurons and glia.

Modulation of Cholesterol Metabolism in Npc1−/− Neurons and Astrocytes by CD—Previous studies in Npc1−/− fibroblasts showed that because cholesterol is sequestered in LE/L, the amount of cholesterol transported to the plasma membrane (5), and ER (6) is reduced. Consequently, NPC1 deficiency increases cholesterol synthesis and uptake in several types of cells (15). We, therefore, assessed cholesterol synthesis in Npc1+/+ and Npc1−/− neurons and astrocytes by incubating the cells for 4 h with [3H]acetate. [3H]acetate incorporation into cholesterol was not increased by NPC1 deficiency in neurons (Fig. 6A) or astrocytes (Fig. 6B). Similarly, we previously observed that NPC1 deficiency did not increase [3H]acetate incorporation into cholesterol in mouse sympathetic neurons.3 As an indication that [3H]acetate uptake and the pool size of the acetate precursor of cholesterol were unaffected by the Npc1 genotype, Fig. 6 demonstrates that radiolabeling of phospholipids in neurons and astrocytes was independent of the Npc1 genotype. Thus, unlike other types of Npc1−/− cells (15), NPC1 deficiency in cerebellar neurons and cortical astrocytes does not appear to enhance cholesterol synthesis. These observations are consistent with the report of Liu et al. (8) who observed no difference between cholesterol synthesis in brains of Npc1+/+ and Npc1−/− mice.

3 B. Karten and J. E. Vance, unpublished data.
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Studies in intact Npc1−/− mice suggest that CD can release cholesterol from LE/L and increase the amount of ER cholesterol available for regulation of cholesterol homeostasis (8, 17). To determine whether CD mobilized cholesterol from LE/L to the ER and decreased cholesterol synthesis, in primary cultures of Npc1−/− neurons and astrocytes, the cells were incubated for 28 h with vehicle alone, or with 0.1 or 1 mM CD; [3H]acetate was included for the final 4 h. In Npc1−/− neurons (Fig. 7A) and astrocytes (Fig. 7C), 0.1 mM CD significantly decreased [3H]cholesterol by 28 and 37%, respectively. In contrast, [3H]cholesterol was not decreased by 0.1 mM CD in Npc1+/− astrocytes (Fig. 7D) and was even slightly increased in Npc1+/+ neurons (Fig. 7B), supporting the hypothesis that 0.1 mM CD increases ER cholesterol in Npc1−/− cells. If, on the other hand, CD had extracted cholesterol from the plasma membrane and ER, one would have expected cholesterol synthesis to be increased. Accordingly, the incorporation of [3H]acetate into cholesterol was increased by 1 mM CD in Npc1−/− and Npc1+/+ neurons (Fig. 7, A and B) and astrocytes (Fig. 7, C and D). Importantly, CD did not significantly alter [3H]acetate incorporation into phospholipids (Fig. 7), implying that [3H]acetate uptake and the intracellular pool size of acetate were not affected by CD. Thus, 0.1 mM and 1 mM CD induce opposite effects on cholesterol synthesis in both neurons and astrocytes.

Cholesterol Esterification in Astrocytes and Neurons—Excess ER cholesterol can be esterified to CE by the ER enzyme acyl-CoA:cholesterol acyltransferase (ACAT) (18). NPC1 deficiency in fibroblasts markedly reduced CE formation via ACAT because the substrate, cholesterol, is trapped in LE/L (6, 15). We, therefore, tested our hypothesis that 0.1 mM CD mobilizes cholesterol from LE/L to the ER in Npc1−/− cells by examining cholesterol esterification in an assay that measures ER cholesterol availability. Npc1−/− and Npc1+/+ astrocytes were incubated with 0.1 or 1 mM CD for 28 h; [14C]oleate ± the ACAT inhibitor Sandoz 58-035 were added for the last 4 h. In Npc1−/− astrocytes, 0.1 mM CD increased [14C]oleate incorporation into CE by 2.6-fold (Fig. 8A); the increase in esterification was abolished by Sandoz 58-035, implying that ACAT was responsible for the increased esterification. These data suggest that 0.1 mM CD increased ER cholesterol. In contrast, 1 mM CD dramatically reduced [14C]CE in Npc1−/− astrocytes (Fig. 8A) consistent with a reduction in ER cholesterol. Moreover, [14C]oleate incorporation into phospholipids of Npc1−/− (Fig. 8B) and Npc1+/+ (Fig. 8D) astrocytes was not significantly different and was unaltered by Sandoz 58-035, indicating that CD did not affect [14C]oleate uptake or the intracellular pool size of oleate. In contrast, in Npc1+/+ astrocytes, neither 1 mM nor 0.1 mM CD significantly altered the amount of [14C]CE (Fig. 8C), consistent with the idea that CD mobilized only small amounts of cholesterol from LE/L to the ER because cholesterol was not sequestered in LE/L of Npc1+/+ cells. These studies provide further evidence that 0.1 mM CD releases cholesterol trapped in LE/L of Npc1−/− astrocytes and increases cholesterol availability at the ER. In addition, the observation that 1 mM CD reduced cholesterol esterification in Npc1−/− astrocytes suggests that 1 mM...
CD extracted cholesterol from the cells and reduced ER cholesterol. Cholesterol esterification was also examined in \( \text{Npc1}^{+/+} \) and \( \text{Npc1}^{-/-} \) neurons. In comparison with astrocytes, very little cholesterol was esterified in cerebellar granule neurons of either \( \text{Npc1} \) genotype (Fig. 8 versus Fig. 9). Moreover, 0.1 mM CD did not increase \(^{14}\text{C}\) CE formation in \( \text{Npc1}^{+/+} \) and \( \text{Npc1}^{-/-} \) neurons was similar, albeit barely detectable (Fig. 9A and B). Consistent with these findings, the level of ACAT1 mRNA in the neurons was only \( \sim 10\% \) of that in astrocytes and did not depend on the \( \text{Npc1} \) genotype (Fig. 9C). We conclude that (i) cholesterol esterification is far less active in cerebellar granule neurons than in...
Expression of Genes of Cholesterol Homeostasis—When the ER cholesterol content of fibroblasts exceeds ~5% of total lipids (6), the expression of genes of cholesterol synthesis and uptake, including SREBP2 and its target genes encoding 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) and LDLR, decreases. In addition, expression of genes that encode two ATP cassette-binding (ABC) proteins involved in cholesterol efflux from cells (ABCA1 and ABCG1) increases. Conversely, when ER cholesterol drops below the 5% threshold, the expression of genes of cholesterol synthesis and uptake increases, whereas mRNAs involved in cholesterol efflux decrease. We, therefore, compared the expression of genes of cholesterol homeostasis in neurons and astrocytes from Npc1+/+ and Npc1−/− mice. In neurons, as in whole brain (14), levels of mRNAs encoding SREBP2, HMGCR and LDLR were not affected significantly by the Npc1 genotype (Fig 10A). However, in astrocytes, SREBP2 and HMGCR mRNAs were reduced by NPC1 deficiency (Fig 10B). Although ABCA1 mRNA was not changed by NPC1 deficiency in either neurons or astrocytes, ABCG1 mRNA was significantly decreased in both neurons and astrocytes (Fig 10, A and B).

We also quantified mRNAs involved in cholesterol metabolism in neurons and astrocytes in response to CD. In Npc1−/− neurons, SREBP2 mRNA was significantly decreased by 0.1 mM CD but increased by 1 mM CD (Fig 11A). Correspondingly, HMGCR mRNA was decreased by 0.1 mM CD and increased by 1 mM CD. In Npc1−/− neurons, mRNAs encoding LDLR, ABCA1 and ABCG1 were not significantly changed by 0.1 mM CD (Fig 11A), whereas 1 mM CD markedly increased LDLR and HMGCR mRNAs and decreased ABCA1 and ABCG1 mRNAs. These observations provide additional support for a model in which 0.1 mM CD mobilizes cholesterol from LE/L to the ER in Npc1−/− neurons. In contrast, in addition to releasing cholesterol from LE/L, the predominant action of 1 mM CD appears to be cholesterol removal from the plasma membrane/ER; thus, the small amount of LE/L cholesterol that reaches the ER appears to be insufficient to compensate for the larger amount of cholesterol removed from the Npc1−/− neurons. In Npc1+/+ neurons, 0.1 mM CD did not alter SREBP2, HMGCR, or LDLR mRNAs, whereas 1 mM CD robustly increased levels of these mRNAs (Fig 11B); furthermore, ABCA1 and ABCG1 mRNAs

FIGURE 6. Incorporation of [3H]acetate into cholesterol of Npc1+/+ and Npc1−/− neurons and astrocytes. Neurons (A) and astrocytes (B) from Npc1+/+ (black) and Npc1−/− (white) mice were cultured for 7 days then incubated with [3H]acetate for 4 h. Lipids were extracted, separated by thin-layer chromatography, and radioactivity was quantified in cholesterol and phospholipids. Data for neurons are means ± S.E. of duplicate measurements from three independent experiments, and for astrocytes, data are means ± S.E. from four independent experiments.

FIGURE 7. CD modulates [3H]acetate incorporation into cholesterol in neurons and astrocytes. Neurons and astrocytes were incubated for 28 h with vehicle (dark gray), 0.1 mM CD (light gray), or 1 mM CD (white); [3H]acetate was included for the final 4 h. Lipids were separated by thin-layer chromatography, and radioactivity was quantified in cholesterol and phospholipids. Data for neurons are means ± S.E. from duplicate determinations of three independent cell preparations. The experiments with Npc1+/+ neurons were performed independently of those with Npc1−/− neurons and therefore are not comparable. For astrocytes, the data are means ± S.E. from four independent cell preparations. The experiments with Npc1+/+ and Npc1−/− astrocytes were performed concurrently. * p < 0.05; ***, p < 0.001.
were markedly attenuated (Fig 11B). Thus, because cholesterol is not sequestered in LE/L of Npc1+/+ cells, it appears that the small amount of cholesterol released from LE/L by 0.1 mM CD was insufficient to raise ER cholesterol content because more cholesterol had been removed from the cell surface.

In Npc1−/− and Npc1+/+ astrocytes, CD altered the levels of mRNAs of cholesterol-responsive genes less profoundly than in neurons (Fig 11, C and D). In Npc1−/− astrocytes, the mRNAs involved in cholesterol accretion (SREBP2, HMGCR, and LDLR) were not changed by 0.1 mM CD, whereas 1 mM CD modestly increased HMGCR and SREBP2 mRNAs (Fig. 11C). In addition, ABCA1 and ABCG1 mRNAs were increased by 0.1 mM but not 1 mM CD (Fig. 11C), suggesting that astrocytes preferentially increase cholesterol efflux in response to 0.1 mM CD.

In Npc1+/+ neurons, ABCA1 and ABCG1 mRNAs were increased by 0.1 mM CD on cholesterol homeostasis appears to be cholesterol extraction from the plasma membrane and depletion of ER cholesterol.

**DISCUSSION**

The remarkable recent findings of Dietschy and co-workers (8, 17, 19) on the benefit of CD in Npc1−/− mice indicate the potential for using CD to treat NPC patients. Because the previous studies were performed in intact mice, we investigated the CD dose response of primary neurons and glial cells from brains of Npc1−/− mice. We show that cholesterol homeostasis in Npc1−/− neurons and astrocytes depends critically on the CD concentration. Although both 1 mM and 0.1 mM CD reduced cholesterol storage in LE/L of Npc1−/− neurons and astrocytes, these CD concentrations exerted opposite effects on cholesterol homeostasis. Thus, 0.1 mM CD released cholesterol trapped in LE/L and increased cholesterol availability at the ER for CE formation as well as for reducing cholesterol synthesis and modulating expression of genes of cholesterol metabolism. On the other hand, 1 mM CD decreased CE formation, reduced mRNAs required for cholesterol efflux and increased cholesterol synthesis and mRNAs involved in cholesterol synthesis and uptake. Therefore, both 0.1 and 1 mM CD mobilized LE/L cholesterol in Npc1−/− cells, the overriding effect of 1 mM CD on cholesterol homeostasis appears to be cholesterol extraction from the plasma membrane and depletion of ER cholesterol.
The response of Npc1\(^{-/-}\) astrocytes and neurons to CD was distinct in several ways. First, 10 mM CD was profoundly toxic to neurons but less deleterious to astrocytes. Second, ACAT-mediated cholesterol esterification was far more active in astrocytes than in neurons but less deleterious to astrocytes. Fourth, 0.1 mM CD increased mRNAs of genes involved in cholesterol efflux in astrocytes but not in neurons. Because glial cells are the major cell type in the brain and play key roles in brain cholesterol metabolism (reviewed in Ref. 20), it was possible that the CD-mediated increase in survival of Npc1\(^{-/-}\) mice/neurons was due to correction of the cholesterol trafficking defect in astrocytes. However, recent studies demonstrate that NPC1 deficiency in neurons rather than astrocytes is the primary factor underlying the neurodegeneration (21, 22).

CD is relatively non-toxic and is currently being administered to some NPC patients. Thus, our studies are timely and imply that the amount of CD to which neurons and astrocytes are exposed is crucial. Continuous exposure of these cells to CD concentrations of 1 mM or higher is likely to be detrimental. Interestingly, despite the half-life of CD in the brain being ~6.5 h, a single injection of 40 mg CD/Kg body weight into Npc1\(^{-/-}\) mouse brains resulted in a brain CD concentration of ~0.1–0.4 mM, which suppressed cholesterol synthesis for >6 days (14). Moreover, the ED\(_{50}\) of CD required for a positive therapeutic effect in the brain is ~0.5 mg/kg. These CD concentrations are similar to the 0.1 mM concentration that was beneficial in the current studies. One limitation to the use of CD for treatment of NPC patients is that transport of CD from the plasma into the brain is inefficient: only ~0.2% of plasma CD enters the brain (14). Thus, because liver disease often occurs in NPC patients, a possible treatment regimen might be simultaneous administration of CD both peripherally and directly into the brain so that CD concentrations in liver and brain would be similar and in the range of 0.1–0.2 mM.

Acknowledgments—We thank Randy Nelson and Russ Watts for excellent technical assistance and Robert B. Campenot and Dennis E. Vance for helpful discussions.

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