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Effects of 2′,3′-Dideoxycytidine and 2′,3′-Dideoxycytidine 5′-Triphosphate on Phospholipid Metabolism in Permeabilized Rat Hepatocytes

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ABSTRACT. Both 2′,3′-dideoxycytidine (ddC) and 2′,3′-dideoxycytidine 5′-triphosphate (ddCTP) inhibit the synthesis of the major phospholipids phosphatidylcholine (PC) and phosphatidylethanolamine (PE) in permeabilized rat hepatocytes. For PC, this appears to be based on competitive inhibition of cholinephosphotransferase (CDPcholine:1,2-diacylglycerol cholinephosphotransferase; EC 2.7.8.2). The study was based on short-term incubations (6–12 min) of the nucleoside/nucleotide analogs with α-toxin permeabilized rat hepatocytes. At a concentration of 1 mM, ddC and ddCTP decreased the incorporation of radiolabelled glycerol-3-phosphate into PC by approximately 50% as compared with control. This was accompanied by a significant increase in diacylglycerol labelling. In the presence of 1 mM CDP-ethanolamine and increasing concentrations of ddC(TP) (0.01–1 mM), the incorporation of radiolabelled glycerol-3-phosphate into PE was decreased to approximately 60% of the control value. When both PC and PE synthesis were operative, the inhibition by ddC(TP) was restricted to PC synthesis. ddC and ddCTP were found to have inhibition constants (K_i) of 496 μM and 452 μM, respectively, for the inhibition of PC synthesis from CDP-choline. Although the inhibitory concentrations of the nucleoside analog and its triphosphate ester are much higher than the *in vivo* plasma concentrations, the possibility is raised that the peripheral neuropathy, seen as a dose-dependent adverse effect of ddC treatment in acquired immunodeficiency syndrome therapy is, at least partly, caused by a perturbation of the phospholipid constitution of neuronal membranes. BIOCHEM PHARMACOL **54**;6:713–719, 1997. © 1997 Elsevier Science Inc.

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Phosphatidylcholine (PC)§ and phosphatidylethanolamine (PE) are major structural compounds of biomembranes and also have an important function in methyl metabolism, lipoprotein metabolism, transmembrane signalling, and cholinergic neurotransmission [1]. The major pathway for *de novo* synthesis of PC is the Kennedy pathway, in which choline is phosphorylated and converted to cytidine diphosphocholine (CDP-choline) by the activities of choline kinase (ATP:cholinephosphotransferase; EC 2.7.1.32) and choline-phosphate cytidylyltransferase (CTP:choline phosphate cytidylyltransferase; EC 2.7.7.15), respectively. CDP-choline, together with diacylglycerol, forms PC and cytidine monophosphate (CMP) in a reaction catalyzed by cholinephosphotransferase (CDP-choline: 1,2-diacylglycerol cholinephosphotransferase; EC 2.7.8.2). PE is synthesized via a similar pathway in which ethanolamine is phosphorylated and converted to cytidine diphosphoethanolamine (CDP-ethanolamine) by the activities of ethanolamine kinase (EC 2.7.1.82) and ethanolamine-phosphate cytidylyltransferase (CTP:ethanolamine-phosphate cytidylyltransferase; EC 2.7.7.14), respectively. CDP-ethanolamine, together with diacylglycerol, forms PE and CMP in a reaction catalyzed by ethanolaminephosphotransferase (CDP-ethanolamine:1,2-diacylglycerol ethanolaminephosphotransferase; EC 2.7.8.1) [2, 3].

2′,3′-Dideoxycytidine (ddC, zalcitabine), an analog of the natural nucleoside 2′-deoxycytidine, is one of the 2′,3′-dideoxynucleoside analogs used for its antiviral activity in the treatment of acquired immunodeficiency syndrome (AIDS) or AIDS-related complex. ddC enters the cell by facilitated diffusion via a nucleoside carrier [4].

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§ *Abbreviations:* ddC, 2′,3′-dideoxycytidine; 3-GP, glycerol-3-phosphate; MOPS, 4-morpholinopropanesulphonic acid; PC, phosphatidylcholine; and PE, phosphatidylethanolamine.

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Following cellular uptake, it can be phosphorylated to the active triphosphate compound 2′,3′-dideoxycytidine 5′-triphosphate (ddCTP), which inhibits human immunodeficiency virus (HIV) reverse transcriptase [5]. The dideoxynucleosides, however, differ from other classes of reverse transcriptase inhibitors in also possessing the ability to act as DNA chain terminators in the triphosphate form. DNA polymerase α, the key enzyme in cellular DNA synthesis, is relatively resistant to the effects of ddCTP, explaining the selective antiviral effect of ddC. However, DNA polymerase β, involved in DNA repair, and in particular mitochondrial DNA polymerase γ are highly susceptible to inhibition by ddCTP, which might account for the adverse effects of ddC observed in clinical trials [6]. The most prominent dose-limiting toxic side effect of ddC is sensory peripheral neuropathy [6, 7]. Although this may be related to the inhibition of DNA polymerases β and γ, it cannot be excluded that ddC has additional antimetabolic effects. Cooney *et al.* [5] have reported that ddC can be converted to 2′,3′-dideoxycytidine diphosphocholine (ddCDP-choline) in ATH8 cells. We have therefore investigated the possibility that ddC and/or its phosphorylated metabolites may interfere with the synthesis of PC and PE. The experiments have been done in α-toxin permeabilized rat hepatocytes, which have proved to be a very useful model for the study of glycerolipid synthesis and its regulation [8, 9]. The permeabilization allows manipulation of the intracellular availability of substrates and other compounds, for which the plasma membrane is normally impermeable, without compromising the integrity of complex enzymatic pathways and organelles. This makes it possible to measure the activity of PC synthesis with CDP-choline as substrate, which is, in fact, a direct *in situ* assay of cholinephosphotransferase. Low molecular weight compounds that will not penetrate an intact cell, e.g. ddCTP, can be tested for their effects on biochemical pathways in permeabilized cells. It can be assumed that the cytosolic concentration of these compounds will be equal to their concentration in the medium within seconds of incubation [8, 9]. Although ddC can penetrate intact cells, we chose to evaluate its effects under the same conditions as ddCTP, i.e. in α-toxin-treated cells. The main disadvantage of permeabilized cells is the fact that their viability is limited in time (see below).

MATERIALS AND METHODS

Materials

[2′,3′-^{3}H]ddC (38.5 Ci/mmol) was obtained from Sigma (St. Louis, MO, USA); the other radiochemicals were from New England Nuclear (Brussels, Belgium). CoA, fatty acid-free BSA, L-glycerol-3-phosphate, and ddCTP were obtained from Boehringer Mannheim (Germany). ddC was a kind gift from Dr. D. G. Johns (National Cancer Institute, National Institutes of Health, Bethesda, MD, USA). Unlabelled palmitic acid, oleic acid, CDP-choline, CDP-ethanolamine, cholinephosphate, CTP, collagenase type IV, and lipid standards for TLC were from Sigma. Glutathione was from Acros Organics (Pittsburgh, PA, USA). Silica gel 60 TLC plates were from Merck (Darmstadt, Germany). The Partisphere SAX HPLC column was from Whatman (Clifton, NJ, USA). The source and quality of other reagents have been described previously [8, 9].

Preparation and Use of α-Toxin

α-Toxin was isolated from the culture fluid of *Staphylococcus aureus* wood 46 strain and purified by a combination of adsorption chromatography (on controlled pore size glass beads) and ion exchange chromatography (on the CM Sepharose cation exchanger) [10, 11]. The concentration of α-toxin in the incubation medium was titrated to obtain minimal leakage of L-lactate dehydrogenase ((S)-Lactate: NAD^+ oxidoreductase; EC 1.1.1.27) from the cells, with maximal incorporation of labelled glycerol-3-phosphate into di- and triacylglycerol. Under these conditions, the hepatocytes remain stable and linearly synthesize glycerolipids for at least 20 min [8].

Animals

Male Wistar rats of approximately 150–200 g were used. The animals were fed a standard pelleted diet *ad libitum* and had unlimited access to water. Rats were starved for 24 h prior to liver excision.

Preparation of Hepatocytes

Hepatocytes were prepared using the collagenase perfusion technique of Zahlten and Stratman [12], modified by EGTA preperfusion according to Seglen [13].

Experiments with Permeabilized Cells

The standard incubation medium (final volume, 1 mL) consisted of 40 mM 4-morpholinopropanesulphonic acid (MOPS), pH 7.2, 10 mM $KHCO_3$, 3% (w/v) Dextran F70, 2% (w/v) fatty acid-free BSA, 5 mM glutathione, 140 mM potassium L-glutamate, 4 mM ATP, 4 mM $MgSO_4$, 4.6 μg of *S. aureus* α-toxin, 0.1 mM CoA, 1 mM long chain fatty acid (palmitate/oleate, 1:1), and 1 mM L-[U-^{14}C]glycerol-3-phosphate (0.8 Ci/mol). Depending on the purpose of the experiment, either CDP-choline (0.2–5 mM) and/or CDP-ethanolamine (1 mM) was added to the incubation medium as substrate for phospholipid synthesis (see "Results"). ddC or ddCTP, dissolved in water, was added at concentrations ranging from 0.01 to 1 mM (see "Results"). The drugs had no acute toxic effect on the cells, as judged from their microscopic appearance and biochemical activity (see below). For some experiments, 1 mM [*Me*-^{14}C]choline phosphate (0.6 Ci/mol) and 0.2 mM CTP were used as substrates for PC synthesis, and radiolabelled glycerol-3-phosphate was replaced by the nonlabelled substrate (1 mM). Incubations were started by adding 2.5×10^6 freshly isolated hepato-

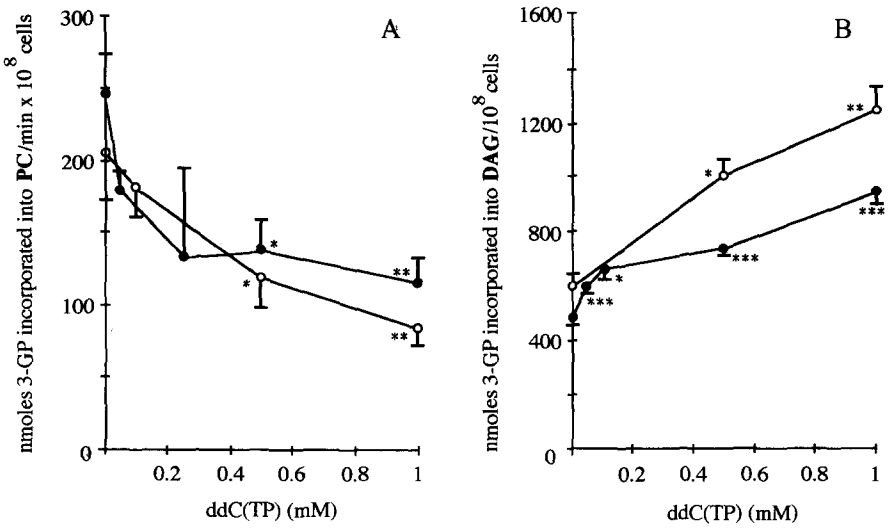

FIG. 1. Effects of ddC (●) and ddCTP (○) on incorporation of radiolabelled 3-GP into PC (A) and diacylglycerol (DAG) (B). Permeabilized cells were incubated with increasing concentrations of ddC or ddCTP, together with labelled 3-GP, fatty acid, and 1 mM CDP-choline in the standard incubation medium (see "Materials and Methods"). A shows the rate of incorporation of label into PC between 6 and 12 min; B shows the incorporation of label into DAG after 12 min of incubation. The results are means ± SEM for at least three independent experiments and are statistically analyzed by two-sample unequal variance *t* test analysis; *, **, and * values are significantly different from the values in the absence of ddC(TP) at the $p < 0.05$, 0.01, and 0.005 levels, respectively.**

cytes; the vials were gassed with O_2:CO_2 (95:5, v/v), capped, and incubated in a gyratory shaker at 37° for 6 or 12 min. Incubations were stopped by adding 5 mL of chloroform:methanol (2:1, v/v), and the incorporation of label into glycerolipids was determined after extraction and purification by TLC. Neutral lipids, lysophosphatidate, and phosphatidate were chromatographed as described earlier [8]. PC and PE were separated on silicagel with chloroform/methanol/25% ammonia (13:7:1, v/v) as solvent system. In the experiments with radiolabelled choline phosphate, incubations were performed as described above, and incorporation of label into PC and (dd)CDP-choline was determined. The water-soluble intermediates of PC synthesis (i.e. (dd)CDP-choline, choline phosphate, and choline) were separated on silicagel with methanol/0.5% NaCl/25% ammonia (10:10:1, v/v) as solvent system. Spots were identified and counted for radioactivity with a Bioscan Imaging System 200 (Bioscan, Washington, DC, USA).

In some experiments, [2',3'-^{3}H]ddC (1 Ci/mol) (concentrations ranging from 0.01 to 1 mM) or unlabelled ddCTP (1 mM) was added to the standard incubation medium, and radiolabelled glycerol-3-phosphate was replaced by the nonradiolabelled substrate. Incubations were performed as described above and were stopped with $HClO_4$ in a final concentration of 3% (w/v).

HPLC Analysis of ddCMP/CMP, ddCDP/CDP, and ddCTP/CTP

After neutralization of the $HClO_4$ extracts with KOH (5 M; approximately 100 μL), the samples were analyzed by anion-exchange HPLC, using a Partisphere SAX column and a buffer system containing 5 mM ammonium dihydrogen phosphate buffer, pH 5.0 (buffer A), and 0.3 M ammonium dihydrogen phosphate buffer, pH 5.0 (buffer B). Elution (flow rate: 2 mL/min) was started with 100% A (5 min), followed by a linear gradient to 100% B (15 min), an isocratic phase with 100% B (20 min), a linear gradient to 100% A (5 min), and re-equilibration with 100% A (5 min). The HPLC apparatus from Waters (Milford, MA, USA) consisted of a model 600 gradient pump, a model 996 photodiode array detector, and a model 717 autosampler, controlled by Millennium software. Identification of the peaks (ddCMP/CMP, ddCDP/CDP, and ddCTP/CTP) was based on retention time and spectral analysis (as compared with commercial standards). Radioactivity in the eluate was determined by the collection of 1-min (2 mL) fractions and liquid scintillation counting after the addition of Highsafe 3 scintillation cocktail from Wallac (Gaithersburg, MD, USA).

RESULTS

We measured the rate of incorporation of radiolabelled glycerol-3-phosphate (3-GP) into PC in the presence of 1 mM CDP-choline and 1 mM fatty acid. When increasing concentrations of ddC were added to the permeabilized hepatocytes, a gradual suppression of PC synthesis occurred. In the presence of 1 mM ddC, the rate was approximately 50% of its control value (Fig. 1A). Correlated with this inhibition was a considerable increase in the level of radiolabelled diacylglycerol (Fig. 1B). The incorporation of labelled 3-GP into the glycerolipid synthesis intermediates lysophosphatidate and phosphatidate and into triacylglycerol was not significantly altered (not shown). This pattern of changes resembles the inhibition of PC synthesis that is seen when the supply of CDP-choline is restricted [8] and can best be explained by an inhibition of cholinephosphotransferase by ddC. Labelled diacylglycerol accumulates because its incorporation into PC is suppressed at the same time as the diacylglycerol acyltransferase (EC 2.3.1.20), which catalyses the conversion of diacylglycerol to triacylglycerol, is saturated with diacylglycerol [8]. The effects on PC synthesis and diacylglycerol labelling were already apparent after 6 min of incubation (not shown), which is very fast in comparison with other studies on ddC metabolism [5, 14]. However, one must bear in mind that the

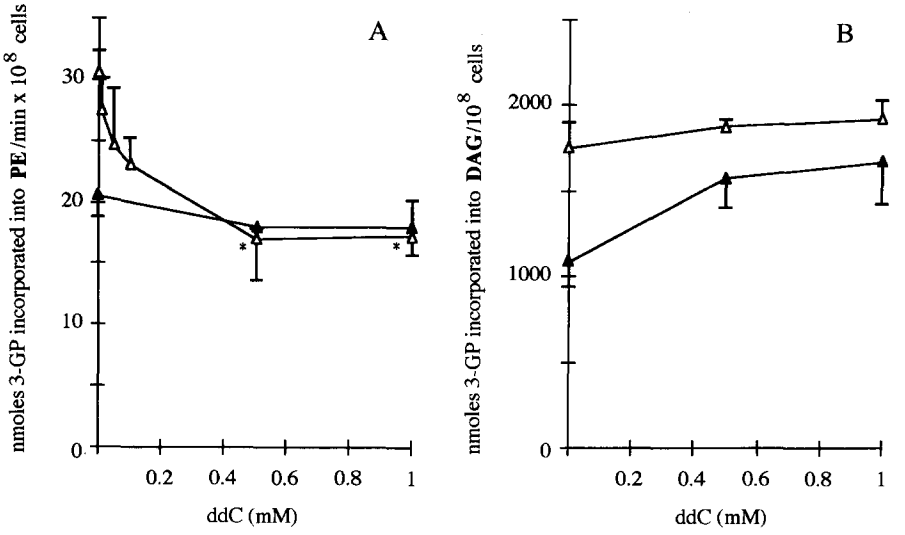

FIG. 2. Effect of ddC on incorporation of radiolabelled 3-GP into PE (A) and diacylglycerol (DAG) (B). Permeabilized cells were incubated with increasing concentrations of ddC, together with labelled 3-GP, fatty acid, and 1 mM CDP-ethanolamine in the standard incubation medium (see "Materials and Methods"). A shows the rate of incorporation of label into PE between 6 and 12 min with (▲) or without (△) the addition of 1 mM CDP-choline; B shows the incorporation of label into DAG after 12 min of incubation with (▲) or without (△) the addition of 1 mM CDP-choline. The results are means ± SEM for at least three independent experiments and are statistically analyzed by two-sample unequal variance *t* test analysis; * values are significantly different from the value in the absence of ddC at a significance level of $p < 0.05$.

cellular uptake of ddC is most probably immediate since the plasma membrane has been permeabilized for low molecular weight compounds.

As ddC is considered to exert its antiviral activity after conversion to its 5′-triphosphate ester (ddCTP), we repeated the experiments with the latter compound. The results were very similar to those obtained with ddC; at a concentration of 1 mM ddCTP the synthesis of PC was inhibited by approximately 50% (Fig. 1A). Again, the decrease in the PC synthesis rate was accompanied by a marked increase in diacylglycerol labelling (Fig. 1B).

To investigate whether the effects of ddC were dependent on its possible conversion to ddCTP, we measured the capacity of the permeabilized rat hepatocytes to phosphorylate the nucleoside analog. Radiolabelled ddC was added to the permeabilized cells in concentrations ranging from 0.01 mM to 1 mM, and, after 6 and 12 min of incubation, the distribution of label in ddC, ddCMP, ddCDP, and ddCTP was determined by HPLC analysis. At least 99% of the radioactivity was present in the ddC fraction, with an overall recovery ranging from 87% to 106%. No ddCTP and ddCDP could be detected, and only 10 μM ddCMP was found after 12 min of incubation with 1 mM ddC. Others [5, 14, 15], using a variety of cultured cells, have reported a limited conversion of ddC to its phosphate esters and another water-soluble metabolite that was tentatively identified as ddCDP-choline by one group [5].

The possible conversion of ddCTP to ddC was also studied by incubating permeabilized hepatocytes with 1 mM unlabelled ddCTP in the routine incubation medium described under "Materials and Methods." After perchloric acid extraction, 91 ± 4% (mean ± SD; $n = 4$) of ddCTP was recovered. No significant amounts of ddCMP or ddCDP were found. Taken together, it appears that ddC and its triphosphate ester are, under the present conditions, not interconverted and that both compounds inhibit, to a similar extent, the last step in the biosynthesis of PC.

In a similar fashion, we studied the effects of ddC on PE synthesis. In a first set of experiments, we substituted CDP-ethanolamine for CDP-choline, thereby preventing PC synthesis. In the presence of 1 mM CDP-ethanolamine, 1 mM radiolabelled 3-GP, and increasing concentrations of ddC (0.01–1 mM), the PE synthesis rate was decreased to approximately 60% of the control value (Fig. 2A). There was only a slight concurrent increase in diacylglycerol labelling (Fig. 2B). The activity of the PE pathway in the absence of ddC was approximately 15% of the activity of the PC pathway, and a partial inhibition of PE biosynthesis was therefore not expected to release much diacylglycerol.

Next, we used the combination of 1 mM CDP-ethanolamine and 1 mM CDP-choline and again examined the effect of ddC. Under these experimental conditions, the PE synthesis rate was only 20 nmol/min × 10^8 cells, as compared with 30 nmol/min × 10^8 cells in the absence of CDP-choline, and, surprisingly, the inhibitory effect of ddC on PE synthesis was suppressed (Fig. 2A). This may indicate that CDP-choline and ddC inhibit PE synthesis via a common mechanism. It has been observed that purified rat liver cholinephosphotransferase and ethanolaminephosphotransferase are competitively inhibited by CDP-ethanolamine and CDP-choline, respectively [16]. When the effect of ddCTP was examined on PE synthesis, equivalent results were obtained (not shown).

To determine the type of inhibition of PC synthesis by ddC and ddCTP, we constructed Dixon plots by measuring the inhibitory effect of ddC, or ddCTP, in the presence of different concentrations of the competitor CDP-choline. These experiments illustrate the unique advantage of the permeabilized cells as compared with intact cells or broken cell preparations; the CDP-choline level cannot be directly manipulated in intact cells, and tissue homogenates or subcellular fractions do not have the capacity to synthesize glycerolipids at normal rates. The results presented in Fig. 3 clearly indicate that both ddC (Fig. 3A) and ddCTP (Fig. 3B) inhibited PC biosynthesis in a competitive fashion. As indicated above, the assay actually measures the activity of

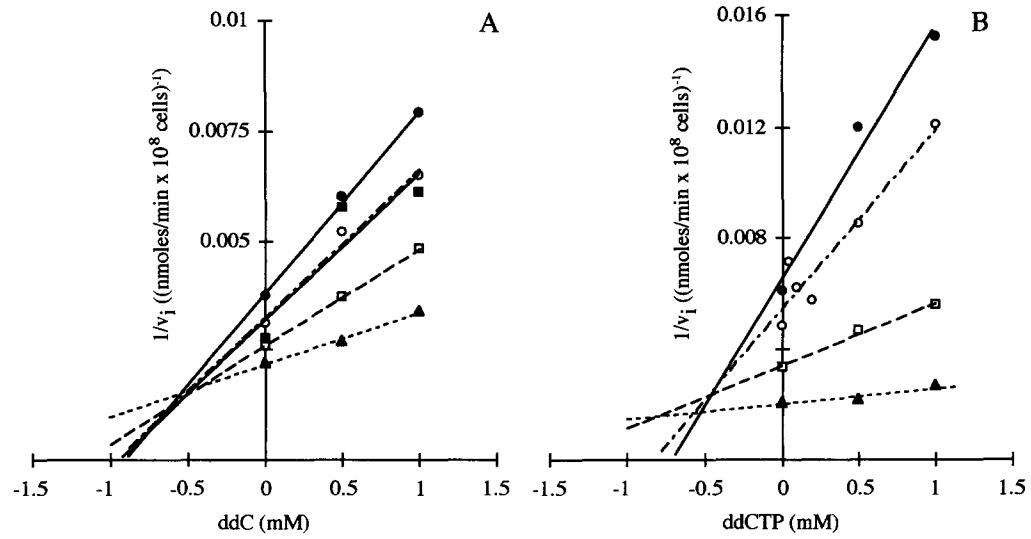

FIG. 3. Dixon plots were constructed from measurements of the inhibitory effect of ddC (A) or ddCTP (B) on cholinephosphotransferase. Permeabilized cells were incubated with increasing concentrations of ddC (0–1 mM) or ddCTP (0–1 mM), together with labelled 3-GP, fatty acid, and different concentrations of the competitor CDP-choline (see "Materials and Methods"). $1/v_i$ is plotted against ddC(TP) concentration (mM). For each concentration of CDP-choline, (i.e. 0.2 mM (●), 0.4 mM (○), 1 mM (■), 2 mM (□), 5 mM (▲)), a straight line is obtained by linear regression (least squares analysis).

cholinephosphotransferase. The K_i values for ddC and ddCTP were found to be 496 ± 65 μM (mean ± SD; n = 5) and 452 ± 20 μM (mean ± SD; n = 3), respectively.

A last set of experiments was performed to see whether ddCTP could interfere with the conversion of cholinephosphate to CDP-choline. This step, catalyzed by cholinephosphate cytidylyltransferase, is considered to limit the rate of PC biosynthesis and is subject to complex physiological regulation [3, 17]. Permeabilized hepatocytes were incubated with 1 mM radiolabelled cholinephosphate and 1 mM ddCTP, and, after 12 min of incubation, the incorporation of label into ddCDP-choline and PC was measured. As shown in Fig. 4, ddCDP-choline was formed, and some label was incorporated into PC, though the latter was not significantly different from the blank in which ddCTP was omitted (not shown). When CTP replaced ddCTP, the incorporation of label into CDP-choline was somewhat lower, but a much higher rate of PC synthesis was obtained, even at a relatively low concentration of 0.2 mM CTP, which was found to be optimal in preliminary experiments (results not shown). When the combination of 1 mM ddCTP and 0.2 mM CTP was tested, PC synthesis was clearly inhibited, as compared with the condition with CTP alone, while the incorporation of label into CDP-choline and ddCDP-choline (the TLC system did not allow us to distinguish them) was similar to the value found with CTP alone. However, the conditions used, i.e. labelled choline phosphate, did not permit us to verify whether ddCTP would still be converted to ddCDP-choline in the presence of CTP. Therefore, the inhibition of PC biosynthesis under the given conditions may be due to decreased CDP-choline supply and/or inhibition of the cholinephosphotransferase by ddCDP-choline, in addition to a direct inhibition of the cholinephosphotransferase by ddCTP (see above).

DISCUSSION

It was shown that the synthesis of PC and PE can be inhibited by ddC, as well as by ddCTP, at the level of the last step of the pathways, catalyzed by the respective

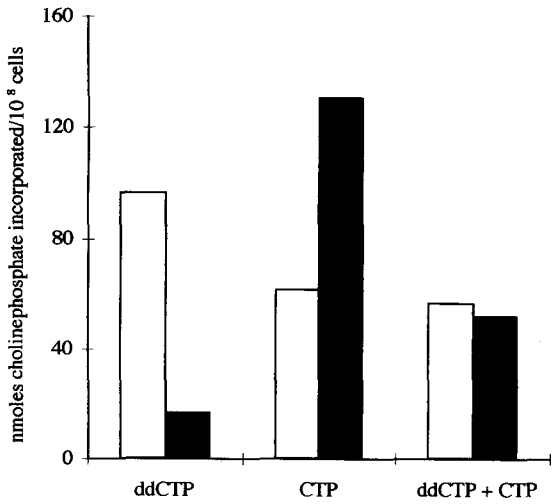

FIG. 4. Effect of ddCTP on cholinephosphate cytidylyltransferase. Permeabilized hepatocytes were incubated with 1 mM ddCTP, 0.2 mM CTP, or the combination of 1 mM ddCTP and 0.2 mM CTP, respectively, together with 1 mM radiolabelled choline phosphate and unlabelled 3-GP (see "Materials and Methods"). Incorporation of label into (dd)CDP-choline (white boxes) and PC (black boxes) was measured after 12 min of incubation. The results are from a representative experiment.

phosphotransferases. The inhibition does not appear to be dependent on conversion of ddC or ddCTP to other 2′,3′-dideoxynucleotides. The very low concentration (i.e. 10 μM) of ddCMP found after incubation with radiolabelled ddC most probably does not account for the inhibitory effect of the latter compound. This consideration is pertinent, since cholinephosphotransferase is believed to catalyze a reversible reaction in which CMP is a reaction product [17]. In separate experiments under similar conditions (results not shown), millimolar concentrations of CMP were needed to obtain 50% inhibition of PC synthesis. When both PC and PE synthesis pathways are operative, the effect of the ddC and its triphosphate ester is mainly restricted to PC synthesis. If this were to occur in tissues *in vivo*, then the phospholipid constitution of the membranes could be affected, possibly leading to a perturbation of membrane functions which in turn might, at least partially, explain the peripheral neuropathy seen in ddC-treated patients. Our experimental setup, i.e. 6–12-min incubations of permeabilized cells, did not allow us to evaluate adverse effects of ddC(TP) on the PC:PE ratio in biological membranes, but the drug might be used as a tool to investigate this biochemical aspect in long term studies. Furthermore, the concentrations at which we see the inhibitory effects of these compounds are much higher than the ddC plasma concentrations that have been measured in individuals treated with the drug [6, 7, 18]. However, it is known that the metabolism of ddC is highly tissue-dependent and that different cell types vary greatly in their ability to take up and metabolize ddC and accumulate the phosphorylated derivatives. Kelley *et al.* [18] reported relatively high tissue levels of ddC in mouse kidney, pancreas, and liver after either a single bolus i.v. injection (100 mg/kg) or a long term i.p. infusion (2 mg/kg/h). The drug mainly appeared in tissues as the parent nucleoside, with its pharmacologically active anabolite (ddCTP) accounting for only a small fraction (<2%) of retained drug. In human T-lymphoblastic cells (Molt 4), ddC accumulated and ddCTP represented 40% of the total acid-soluble pool of ddC metabolites [19]. Studies of ddC anabolism were also carried out by Cooney *et al.* [5] with a panel of human, murine, and caprine cell lines of both lymphoid and non-lymphoid origin. The ability to activate ddC was demonstrated in all lines examined, except for a cell line deficient in deoxycytidine kinase, the enzyme responsible for the phosphorylation of ddC to ddCMP. After addition of 1 mM ddC to murine leukemia L1210 cells, Balzarini *et al.* [20] reported ddCDP and ddCTP intracellular levels of 55 μM and 68 μM, respectively. Others [21] studied nucleoside permeation and metabolism in lymphocytes, macrophages, and bone marrow cells isolated from healthy human volunteers and found no ddC or ddCTP accumulation inside the cells. Even the antiviral activity of ddC is target cell-dependent: although relatively low doses of ddC were adequate to prevent feline immunodeficiency virus infection of feline lymphoid cells, 8- to 80-fold higher doses were needed to block infection of bone marrow cells [22]. Cook and Spence [15], reporting on the effects of ddC on cell proliferation and PC biosynthesis in tumor lines of nervous system origin, could not find any specific changes but cautioned against the extrapolation of their observations to normal cells. Clearly, the same discretion must be applied to our results, but the possibility is raised, for the first time, that the toxicity of ddC may at least partly be related to a perturbation of cellular phospholipid metabolism.

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