Trifluoperazine and other anaesthetics inhibit rat liver CTP : phosphocholine cytidylyltransferase

Steven L. Pelech, Fateem Jetha and Dennis E. Vance*

Department of Biochemistry, University of British Columbia, Vancouver, British Columbia, V6T 1W5 Canada

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Chlorpromazine (25 μM) and trifluoperazine (25 μM) inhibited by 5-fold the activity of CTP : phosphocholine cytidylyltransferase, the rate-limiting enzyme for phosphatidylcholine biosynthesis, in rat liver cytosol. Addition of saturating amounts of rat liver phospholipid to the enzyme assay rapidly reversed the drug-mediated inhibition. Three-fold or greater concentrations of these drugs were required to produce a 50% inhibition of the microsomal cytidylyltransferase. Incubation of rat hepatocytes with 20 μM trifluoperazine or chlorpromazine did not inhibit phosphatidylcholine biosynthesis. These results provide additional evidence for the hypothesis that the active form of cytidylyltransferase is on the endoplasmic reticulum and the enzyme in cytosol appears to be latent.

Phosphatidylcholine biosynthesis *Rat hepatocyte* *Protein translocation*

1. INTRODUCTION

The rate-limiting reaction of the CDP-choline pathway for phosphatidylcholine synthesis in rat liver is catalyzed by the regulatory enzyme, CTP : phosphocholine cytidylyltransferase [1]. This enzyme exists in soluble and membrane-bound forms which are interconvertible [2]. Although >80% of the rat liver cytidylyltransferase is cytosolic [3], recent studies have correlated the rate of phosphatidylcholine biosynthesis with the amount of microsomal enzyme activity [4–6]. The microsomal cytidylyltransferase is fully active in the absence of exogenous phospholipid liposomes [7]. By contrast, the soluble enzyme purified 6-fold from cytosol by Sepharose 6B chromatography has a low basal activity but can be stimulated 50-fold by phospholipid liposomes [7]. Consequently, the hypothesis has developed that cytosol functions as a reservoir of inactive cytidylyltransferase which can be activated after translocation to the endoplasmic reticulum [6,8,9].

Here, we have exploited the ability of various anaesthetics to interact with phospholipids [10,11] to demonstrate that membrane phospholipids which contaminate cytosol may be responsible for the detectable cytidylyltransferase activity in this fraction. Although cytosolic cytidylyltransferase was extremely sensitive to inhibition by trifluoperazine, chlorpromazine and tetracaine, the microsomal enzyme was more resistant. Furthermore, the rate of the cytidylyltransferase-catalyzed reaction in monolayer cultures of rat hepatocytes was not substantially inhibited by the antipsychotic drugs, consistent with the notion that the microsomal cytidylyltransferase is largely responsible for CDP-choline production.

2. MATERIALS AND METHODS

Trifluoperazine was the generous gift of Smith, Kline and French. All other antipsychotic drugs were purchased from Sigma. Cytosol and microsomes were prepared from rat liver homogenized in 0.145 M NaCl as in [12]. Cytidylyltransferase (cytosolic protein, 1.5 mg or microsomal protein,

* To whom correspondence should be addressed

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1.0 mg) was preincubated in 200 μl with Tris–succinate (pH 6.5) 20 μmol and drug, 0–240 μmol. The preincubation commenced with the addition of cytosol or microsomes at 37°C. After 10 min, 50 μl were removed and added to 50 μl of assay mixture which contained [*methyl*-^{3}H]phosphocholine (15 μCi/μmol), 150 nmol; CPT, 200 nmol and magnesium acetate, 750 nmol. For some incubations, the assay mixture was resuspended in phospholipid (6 mg/ml) prepared from total rat liver [13]. After an additional 8 (microsomes) or 15 min (cytosol) at 37°C, the reaction was terminated by immersion of the reaction tube into boiling water for 2 min. Radioactivity incorporated into CDP-choline was determined as in [12].

Hepatocytes were isolated from female Wistar rats (125 g) by a collagenase perfusion technique [14] and cultured in 60 mm diam. plastic culture dishes (LUX Contur) as in [1]. Twenty hours after plating, hepatocytes were incubated for 2 h in serum-free medium (arginine-free Dulbecco's modified Eagle's medium with 28 μM choline chloride, 100 nM insulin, 0.4 mM ornithine, 100 μg sulphate streptomycin/ml, 100 units/ml penicillin G and 10 mM Hepes [4-(2-hydroxyethyl)-1-piperazine–ethane sulphonic acid], pH 7.4). Subsequently, the medium was removed and the cells were pulsed for 30 min with 15 μCi [*methyl*-^{3}H]choline (0.18 Ci/mmol) dissolved in medium. Afterwards the cells were incubated for 90 min in unlabeled medium in the presence of 0–100 μM drug. At the end of the chase period, the cells were harvested and the radioactivity quantitated in the various choline metabolites after thin-layer chromatography [1].

3. RESULTS AND DISCUSSION

The influence of various anaesthetics on the activity of soluble cytidylyltransferase was assessed after a 10 min preincubation of the cytosolic enzyme with the drugs (fig.1). Chlorpromazine and trifluoperazine (25 μM) reduced the cytidylyltransferase activity 5-fold, while tetracaine (50 μM) produced 50% inhibition (fig.1, left). Moderate inhibition (28%) resulted after incubation with dibucaine or propranolol (50 μM), but lidocaine and procaine were without effect. Preincubation of the cytosolic enzyme with trifluoperazine or chlorpromazine was not required for expression of the inhibitory effects of these drugs (not shown).

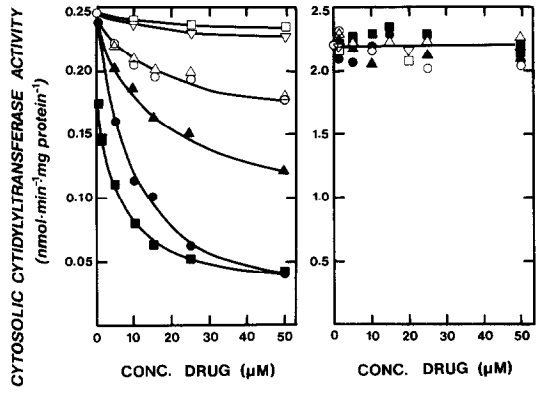

Fig.1. Effect of anaesthetic drugs on rat liver cytosolic cytidylyltransferase. Cytidylyltransferase activity in cytosol was determined in the presence of various drugs (0–50 μM) as in section 2. *Left:* absence of phospholipid liposomes; *right:* presence of phospholipid liposomes in the assay. Specific enzyme activity: chlorpromazine (■); dibucaine (△); lidocaine (□); procaine (▽); D,L-propranolol (○); tetracaine (▲); trifluoperazine (●). This experiment was repeated twice with similar results.

Studies with the calcium-activated, phospholipid-dependent protein kinase (protein kinase C) have established that inhibition of the kinase by anaesthetic drugs is a consequence of interaction of these drugs with phospholipids [15–17]. Like protein kinase C, cytidylyltransferase can be dramatically stimulated by phospholipid liposomes [7]. Specifically, rat liver cytosolic cytidylyltransferase is activated by phosphatidylserine, phosphatidylinositol, phosphatidylglycerol and lysophosphatidylethanolamine [7]. Cytosolic cytidylyltransferase activity was stimulated 9-fold by total rat liver phospholipid (compare activities without drugs in fig.1, left and right). Furthermore, addition of saturating amounts of phospholipid in the enzyme assay rapidly reversed any inhibition of cytidylyltransferase activity by the antipsychotic drugs during the preincubation (fig.1, right). Hence, the observed inhibition of cytosolic cytidylyltransferase in the absence of added phospholipid by the drugs may have arisen from the interactions of the drugs with endogenous phospholipid [10,11] which activate the enzyme. Alternatively, the drugs may have competitively bound to the sites where the phospholipids interact for activation of the cytidylyltransferase. This latter possi-

bility seems less likely since drugs with different structures inhibited the cytosolic cytidylyltransferase.

Since the inhibitory action of the compounds on the cytosolic cytidylyltransferase activity seemed to be reversed by phospholipid, the microsomal cytidylyltransferase might be more resistant to inhibition by these compounds due to a phospholipid rich environment. As shown in fig.2, ⩾3-fold concentrations of drug were required for 50% inhibition of microsomal cytidylyltransferase than needed for an equivalent reduction of the cytosolic enzyme activity. Moreover, the inhibition of the microsomal cytidylyltransferase by 30 μM trifluoperazine was abolished by inclusion of exogenous phospholipid (not shown) or by increasing the concentration of microsomes used in the enzyme assay (fig.3).

If the membrane-bound form of cytidylyltransferase represents the active species of this enzyme in rat hepatocytes and this form is relatively resistant to inhibition by some of these drugs, then these compounds should not produce reduced rates of phosphatidylcholine synthesis in intact cells. This hypothesis was tested in rat hepatocytes which had been pulsed for 30 min with [*methyl*-^{3}H]choline. At the end of the pulse period, 90% of the label that had not been oxidized to betaine was associated with the phosphocholine pool of the cells (not shown). The presence of the drugs in the chase medium did not appreciably reduce the rate of incorporation of label from phosphocholine into phosphatidylcholine after 90 min (table 1). On the other hand, the appearance of label into phosphatidylcholine was slightly stimulated (1.3-fold, $p<0.05$) by tetracaine or dibucaine (100 μM). Since the specific radioactivity of [*methyl*-^{3}H]phosphocholine was the same in all cells at the start of the chase, any changes in the labeling of phosphatidylcholine induced by these drugs would have reflected actual alterations in the rate of the cytidylyltransferase-catalyzed reaction and phosphatidylcholine synthesis. These findings illustrate the potential difficulties in translating the effects of compounds on enzymes in vitro to in vivo situations.

The inhibitory effects of the drugs on rat liver cytidylyltransferase can be attributed to the ability of these compounds to compete with, or for, phos-

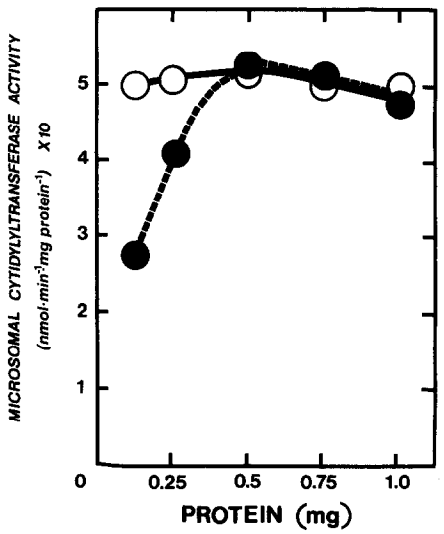

Fig.3. Reversal of trifluoperazine inhibition of microsomal cytidylyltransferase activity by increasing [protein]. Microsomal cytidylyltransferase was incubated for 3–8 min at 37°C in 100 μl with Tris–succinate (pH 6.5), 7.5 μmol; magnesium acetate, 750 nmol; [*methyl*-^{3}H]-phosphocholine (15 μCi/μmol), 150 nmol; CTP, 200 nmol and 0.1–1 mg microsomal protein in the absence (○) or presence (●) of trifluoperazine, 3 nmol.

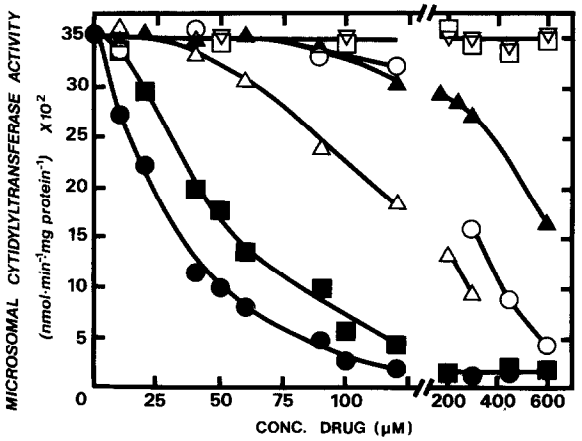

Fig.2. Effect of antipsychotic drugs on rat liver microsomal cytidylyltransferase. Cytidylyltransferase activity in microsomes was determined in the presence of various drugs (0–600 μM) as in section 2. Specific enzyme activity: chlorpromazine (■); dibucaine (△); lidocaine (□); procaine (▽); D,L-propranolol (○); tetracaine (▲); trifluoperazine (●). This experiment was repeated 3 times with similar results.

Table 1

Effect of antipsychotic drugs on phosphatidylcholine synthesis in cultured rat hepatocytes.

	Phosphatidylcholine[a] (dmp × 10^{-5}/dish)	Control (%)
Control	1.37 ± 0.03	–
Trifluoperazine (20 μM)	1.24 ± 0.10	91
Chlorpromazine (20 μM)	1.19 ± 0.03	87
D,L-Propranolol (100 μM)	1.50 ± 0.10	109
Procaine (100 μM)	1.39 ± 0.16	101
Tetracaine (100 μM)	1.76 ± 0.13	128
Dibucaine (100 μM)	1.80 ± 0.13	131
Lidocaine (100 μM)	1.27 ± 0.08	93

[a] Incorporation into phosphatidylcholine during a 90 min chase with hepatocytes which were prelabeled with [*methyl*-^{3}H]choline. The incorporation into phosphatidylcholine during the pulse period (6.3×10^4 dpm/dish) has been subtracted.

Drugs were introduced in the chase medium of cells which were prelabeled with 15 μCi of [*methyl*-^{3}H]choline (0.18 Ci/nmol) for 30 min. Each value is the mean of three dishes, and the standard deviation is given

pholipids which stimulate the enzyme. These drugs, however, have a multitude of effects, including antagonism of calmodulin action [18]. Cytidylyltransferase appears to be regulated by protein phosphorylation [12], and it is possible that this enzyme may be influenced by calmodulin-dependent protein phosphorylation. However, we would expect that an inhibition of calmodulin-dependent phosphorylation by these drugs would have resulted in an activation rather than an inhibition of cytidylyltransferase [12]. The findings with cytidylyltransferase and protein kinase C [15–17] demonstrate that caution must be exercised if anaesthetics are used exclusively to probe for calmodulin-sensitivity of enzymes which associate with membranes.

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