# Cationic amphiphilic drugs inhibit the synthesis of long-chain fatty acyl coenzyme A in rat brain microsomes

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The effect of cationic amphiphilic drugs (CAD) on the synthesis of thiol esters of coenzyme A with long-chain fatty acids was studied in microsomes of rat brain in vitro. The results indicate that propranolol, tetracaine and to a lesser extent, chloroquine, inhibit enzyme activity. Procaine and lidocaine did not inhibit enzyme activity in concentrations up to 0.8 mM. This inhibition seems to be directed primarily to the synthesis of polyunsaturated fatty acyl coenzyme A. The results also suggest that this inhibition may be due to the action of CAD on the microsomal membrane and not to an interaction of these drugs with the fatty acid substrates.

*Propranolol* *Tetracaine* *Chloroquine* *Arachidonic acid* *Docosahexanoic acid* *Long-chain acyl coenzyme A synthetase* *Local anesthetic*

## 1. INTRODUCTION

Cationic amphiphilic drugs (CAD) alter the metabolism [1–9] and topology [10] of phospholipids and cause functional impairment of excitable membranes. These drugs inhibit impulse conduction as a result of an effect on the sodium channel [11–13]. Among the effects of CAD on membrane lipid metabolism is the redirecting of the de novo biosynthesis of glycerolipids by the inhibition of phosphatidate phosphohydrolase [8,14–16]. CAD also inhibit the activities of (a) acyl coenzyme A (CoA):lysolecithin acyltransferase of rat liver microsomes [17], (b) acyl-CoA:cholesterol acyl transferase of rat and rabbit arterial microsomes [18–20] and (c) phospholipase A from various sources [8,21–25]. Several other steps in lipid metabolism also are affected, as has been shown in labeling studies with various tissues [5,8,26–30]. However, there is no information about the effect of CAD on the formation of thiol esters of long-chain fatty acids with CoA. This reaction may play a role in the tenacious retention of docosahexaenoic acid (22:6) by nervous tissue [31] and in limiting the availability of free arachidonic acid (20:4) for the formation of prostaglandins and lipoxygenase-leukotriene products [32]. The present investigations evaluated the effect of propranolol, chloroquine and local anesthetics on the microsomal fatty acid-activating enzyme system in rat brain in vitro using labeled palmitic acid (16:0), 20:4 and 22:6 substrates.

## 2. MATERIALS AND METHODS

Male albino Wistar rats weighing 200–250 g were purchased from Harlan Sprague-Dawley, Indianapolis, IN. Tetracaine HCl, procaine HCl, lidocaine and chloroquine diphosphate salt were purchased from Sigma, St Louis, MO. Propranolol HCl was from Ayerst Labs, New York, NY. [1-$^{14}$C]Palmitic acid (54 mCi/mmol), all-*cis*-5,8,11,14-[1-$^{14}$C]arachidonic acid (58 mCi/mmol) and all-*cis*-4,7,10,13,16,19-[U-$^{14}$C]docosahexaenoic acid (160 mCi/mmol) were purchased from New England Nuclear, Boston, MA. The sources of all other chemicals used in the present investigation have been given previously [31].

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Rats were decapitated, brains removed and homogenized, and microsomes were isolated as in [31]. Drugs were dissolved in 10 mM Tris-HCl buffer, pH 8.0, and added to the incubation system wherever needed. The fatty acid activation reaction has been described [31]. The assay mixture contained 30 $\mu$mol Tris-HCl (pH 8.0), 4 $\mu$mol $MgCl_2$, 500 nmol ATP, 200 nmol dithiothreitol, 100 nmol CoA, 5 nmol labeled fatty acid and 60–70 $\mu$g microsomal protein in a final volume of 0.2 ml. The reaction was carried out for 5 min at 37°C. One nmol of each fatty acid contained 32000–43000 dpm. Protein content of microsomes was estimated by the method of Lowry et al. [33] using crystalline bovine serum albumin as a standard.

## 3. RESULTS AND DISCUSSION

When brain microsomal fractions were preincubated with cationic amphiphilic drugs an inhibition in the synthesis of CoA thiol esters of 16:0, 20:4 and 22:6 was found. The inhibitory effect was concentration-dependent and propranolol was the most potent inhibitor, followed by tetracaine and chloroquine. Procaine and lidocaine did not inhibit fatty acid activation under comparable conditions up to 0.8 mM (fig.1). These two local anesthetics inhibited acyl transferase activity, but required 20–30-fold higher concentrations than that of tetracaine [17]. Similar observations on the esterification of oleate to cholesterol have been made with rabbit aorta microsomes [18,20].

The concentration of CAD required to inhibit the reaction by 50% varied with the substrate used (table 1). The amount of propranolol needed was much lower than that of tetracaine or chloroquine. The activation of polyunsaturated fatty acids, especially arachidonic acid, seems to be more susceptible to CAD than palmitic acid except for chloroquine.

Studies were extended to evaluate the mechanism by which CAD inhibits the fatty acid activation enzyme system (table 2). When the drugs were added (100–400 $\mu$M) to the incubation medium prior to the onset of the reaction, there was no inhibition in thiol ester formation. Similar results were obtained when the drugs were preincubated with fatty acids (table 2). However, there

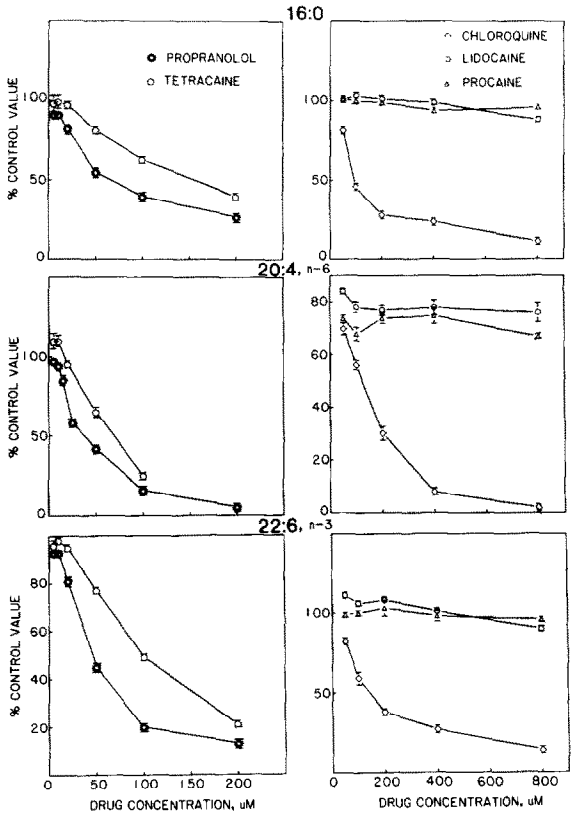


Fig.1. Effect of varying amounts of CAD on the fatty acid activating enzyme system from rat brain microsomal membrane. Different concentrations of drug were preincubated with 60–70 $\mu$g microsomal membrane protein at 37°C for 5 min; control tubes were preincubated with buffer. After this preincubation, the mixture of assay ingredients was added and incubated 5 min at 37°C. The reaction was terminated and the labeled fatty acyl CoA formed was extracted and assayed as described previously [32]. Each value represents mean ± SE of at least 6 incubations carried out at two or three different times with the various microsomal preparations. Details of the assay system are described in section 2. The mean ± SE of control values (nmol/min per mg protein) obtained for the fatty acid activating enzyme system with labeled palmitate, arachidonate and docosahexaenoate were 5.6 ± 0.18 ($n$ = 8), 5.93 ± 0.12 ($n$ = 10) and 1.93 ± 0.05 ($n$ = 8), respectively.

was a significant inhibition when microsomes were preincubated with the drugs. These results suggest that (a) preincubation of the drug with microsomal fraction is necessary to inhibit fatty acid activation

Table 1

Concentration of cationic amphiphilic drugs required to produce 50% inhibition in microsomal fatty acid activation

| Drug | Fatty acid ($\mu$M) | | |
|---|---|---|---|
| | 16:0 | 20:4 | 22:6 |
| Number of observations | 8 | 10 | 8 |
| Propranolol | 71 ± 2.1 | 35 ± 1.5 | 45 ± 2.4 |
| Tetracaine | 159 ± 4.2 | 70 ± 1.2 | 102 ± 2.8 |
| Chloroquine | 93 ± 3.2 | 117 ± 4.4 | 142 ± 4.8 |

Details of the assay system and other conditions are described in section 2. The values were calculated from data in fig.1

acid activation enzyme system by disrupting the membrane structure and thereby inactivating the enzyme and/or by interacting with the active site of the enzyme. The various drug concentrations needed to produce a 50% inhibition of enzyme activity with the different fatty acid substrates used suggest that both of the above possibilities may be involved in this inhibition.

## ACKNOWLEDGEMENT

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Table 2

Effect of cationic amphiphilic drugs on fatty acid activation in rat brain microsomes

| Pre-incubation (5 min) | Incubation (5 min) | Control[a] | | Drugs[b] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Propranolol | | | Tetracaine | | Chloroquine | | |
| | | | | 100 $\mu$M | | 200 $\mu$M | 200 $\mu$M | | 400 $\mu$M | | |
| | | 16:0 | 20:4 | 16:0 | 20:4 | 20:4 | 16:0 | 20:4 | 16:0 | 20:4 | |
| None | Complete assay mixture plus drug | 7.8 ± 0.19 | 9.5 ± 0.17 | 97 ± 1.5 | 99 ± 2.2 | 102 ± 2.4 | 96 ± 1.7 | 99 ± 2.2 | 97 ± 1.4 | 99 ± 2.4 | |
| Fatty acid plus drug | Addition of other components of assay mixture | 7.7 ± 0.14 | 9.4 ± 0.20 | 98 ± 1.1 | 96 ± 1.6 | 102 ± 3.3 | 100 ± 2.3 | 99 ± 1.1 | 103 ± 2.0 | 102 ± 3.9 | |
| Microsomes plus drug | Addition of other components of assay mixture | 5.5 ± 0.10 | 5.4 ± 0.13 | 40 ± 0.7 | 16 ± 0.3 | 5.4 ± 1.4 | 40 ± 1.0 | 2.4 ± 0.6 | 24 ± 0.5 | 8 ± 1.0 | |

[a] Values expressed as nmol/min per mg protein and are mean ± SE, $n$ = 6–8
[b] Values expressed as % control and are mean ± SE, $n$ = 6–8

Details of assay system and other conditions are described in section 2

and (b) there is no drug effect on the membrane when these drugs are added in the presence of other assay components (perhaps because of the protective effects of one or more of the assay mixture components).

These results suggest that CAD inhibit the fatty

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