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Inhibition of Hepatic Microsomal Cytochrome P-450-Dependent Monooxygenase Reactions by Fatty Acyl CoA

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SUMMARY

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A study was undertaken to determine the mechanism by which fatty acyl CoA (stearoyl or oleoyl CoA) inhibits NADPH-supported hepatic cytochrome P-450-dependent monooxygenase systems. About half of the inhibitory effect of fatty acyl CoA on ethylmorphine N-demethylase activity, and all of the inhibitory effect on aniline p-hydroxylase activity, was shown to be due to stimulation of lipid peroxidation. The remaining half of the inhibition of ethylmorphine demethylation was shown not to be due to a) competition for electrons from NADPH by the fatty acyl CoA desaturase system, b) inhibition of NADPH-cytochrome c reductase or NADPH-cytochrome P-450 reductase, c) 3′5′ ADP, which might have been formed from CoA through the action of nucleotide pyrophosphatase, d) a shortage of electrons from NADPH due to an inhibitory effect of fatty acyl CoA on glucose 6-phosphate dehydrogenase, or e) fatty acyl CoA or its products acting as substrate inhibitors. Stearoyl CoA prevented the stimulation of NADPH-cytochrome P-450 reductase by ethylmorphine and caused a small but consistent loss of the type I binding spectrum elicited by ethylmorphine. These observations and the kinetics of the inhibition of ethylmorphine N-demethylase by stearoyl CoA suggest that fatty acyl CoA may inhibit hepatic monooxygenase reactions by acting as a detergent.

INTRODUCTION

In a previous publication from this laboratory (1) it was shown that stearoyl CoA inhibited NADPH-supported microsomal ethylmorphine N-demethylase activity. When synergised by NADH, the reaction was inhibited to the same degree. Cyanide reversed inhibition of the synergised reaction completely, but restored only about 25% of the lost activity when NADPH was the sole source of electrons. Inhibition of the synergised reaction could be explained, at least in part, if activation of the cyanide sensitive desaturation system by stearoyl CoA caused electrons from NADH to be diverted from the cytochrome P-450-dependent demethylase system via cytochrome b_5, which serves both systems. However, this mechanism could not explain the inhibitory effect on demethylase activity when NADPH was the only source of electrons because only a relatively small percentage of the inhibition was reversed by cyanide. Recently, Montgomery and Cinti (2) described an experiment which cast further doubt on the view that activation of the desaturase system could play an important role in the inhibition of non-synergized cytochrome P-450-dependent

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monooxygenase systems by stearoyl CoA. They observed that benzo[a]pyrene hydroxylation by kidney cortex microsomes was inhibited by stearoyl CoA even though stearoyl CoA was not desaturated by these microsomes. They also observed that p-hydroxylation of aniline was inhibited by more than 90% by a concentration of stearoyl CoA which inhibited ethylmorphine N-demethylase by only about 10%, a discrepancy that is difficult to reconcile with a mechanism which invokes a shunting of electrons from NADPH to the desaturase system. The questions raised by these experiments prompted the current study of the inhibition of microsomal drug metabolism by fatty acyl CoA.

MATERIALS AND METHODS

Stearoyl CoA, oleoyl CoA, CoA and Na stearate were purchased from Sigma Chemical Company. Male Simonson Bio-Lab rats (200–250 g) were used. Hepatic microsomes, prepared as described previously (3), were used on the day of preparation. Microsomal aniline p-hydroxylase activity was determined by the method of Brodie and Axelrod (4) as modified by Kato and Gillette (5), which uses ether extraction, or by the method of Imai *et al.* (6), which uses TCA precipitation. The same medium as that described previously (3) was used except that EDTA (50 μM) was added. Microsomal ethylmorphine N-demethylase activity was determined by the Nash method (7) as described previously (3) except that Mg^{++} was omitted from the medium and EDTA was added unless stipulated otherwise. NADPH and $NADP^+$ were determined by the spectrophotometric methods described by Klingenberg (8). NADPH-cytochrome c and NADPH cytochrome P-450 reductase activities were assayed as described by Williams and Kamin (9) and by Gigon *et al.* (10), respectively. Lipid peroxidase activity was estimated by measuring the amount of malondialdehyde formed (11). Apparent kinetic constants were calculated by the method of Wilkinson (12).

RESULTS

Effect of magnesium on microsomal ethylmorphine N-demethylation and lipid peroxidation. Cooper and Brodie (13) observed that Mg^{++} enhanced the microsomal metabolism of hexobarbital, and since then, the cation has been added routinely to incubation media by most investigators when monooxygenase systems have been studied. We observed early during the current studies that Mg^{++} precipitates stearoyl CoA and that when sufficient Mg^{++} was added to the incubation medium, the inhibitory effect of stearoyl CoA on ethylmorphine N-demethylation was reversed. This complication raised the question of the feasibility of omitting Mg^{++} from the incubation medium. Peters and Fouts (14) observed that the metabolism of certain substrates was not enhanced by commonly used concentrations of Mg^{++}; in fact, inhibition occurred in certain cases. Ethylmorphine was not one of the substrates used by these investigators. A study of the effect of Mg^{++} on ethylmorphine N-demethylation was therefore undertaken. Lipid peroxidation depresses monooxygenase activity (15–17). The possibility was considered that Mg^{++} might "stimulate" monooxygenase activity by inhibiting lipid peroxidation. Accordingly, the effect of Mg^{++} (2.0 mM) on ethylmorphine N-demethylation was determined in the absence and the presence of 0.05 mM EDTA. This is a concentration of EDTA which will inhibit lipid peroxidation almost completely without lowering the concentration of Mg^{++} appreciably. Results are summarized in Table 1. The magnitude of the stimulatory effect of 2.0 mM Mg^{++} on ethylmorphine N-demethylation was seen to depend upon the kind of buffer used; in the absence of EDTA a 30% stimulation was observed with phosphate buffer, but a 50% stimulation was seen with Tris buffer. The reason for this difference is not known. When phosphate buffer was used and EDTA was present, Mg^{++} had no significant effect on ethylmorphine N-demethylation. When phosphate buffer was used, Mg^{++} caused a 30% reduction in lipid peroxidation. This may have contributed to the 30% increase in demethylation observed in the absence of EDTA. Much of this increase would appear to be due to the prevention of lipid peroxidation. The stimulatory effect of Mg^{++} on demethylation

TABLE 1

Effect of Mg^{++} on microsomal ethylmorphine (EM) N-demethylation and lipid peroxidation

The incubation medium (5 ml, pH 7.4) contained phosphate or tris buffer (40 mM), semicarbazide (7.5 mM), glucose 6-phosphate (4 mM), $NADP^+$ (0.4 mM), glucose 6-phosphate dehydrogenase (0.4 units/ml), EM (2 mM) and microsomal protein (0.6 mg/ml). Incubation time; 10 min (in air). Values are the mean ± SE of results obtained in 4 experiments.

Additions	EM N-demethylation[a]		Lipid peroxidation[b]	
	PO_4^- buffer	Tris buffer	PO_4^- buffer	Tris buffer
None	4.80 ± 0.63 (100)	6.42 ± 0.82 (100)	8.21 ± 1.09 (100)	3.01 ± 0.71 (100)
Mg^{++} (2 mM)	6.29 ± 0.71 (131)	9.78 ± 0.73 (152)	5.76 ± 0.10 (70)	2.95 ± 0.58 (98)
EDTA (50 μM)	6.75 ± 0.76 (141)	6.83 ± 0.72 (106)	1.08 ± 0.38 (13)	1.41 ± 0.38 (47)
Mg^{++} (2 mM) + EDTA (50 μM)	7.31 ± 0.96 (152)	10.00 ± 1.08 (156)	1.28 ± 0.32 (16)	1.53 ± 0.19 (51)

[a] nmoles HCHO formed/mg of protein/min.

[b] malondialdehyde formation: nmoles/mg of protein/10 min.

when Tris buffer was used occurred both in the presence and absence of EDTA and appeared to be unrelated to lipid peroxidation, which is minimal with this buffer because of its low iron content.

In view of these findings, Mg^{++} was omitted in subsequent experiments and phosphate buffer was used except when a low rate of lipid peroxidation was sought without adding EDTA, in which cases Tris was used.

Effects of stearoyl CoA, oleoyl CoA, CoA and stearate on the recovery of products of aniline hydroxylation and ethylmorphine N-demethylation. Chhabra *et al.* (18) showed that cysteine inhibits the recovery of p-aminophenol when aniline p-hydroxylase is assayed by the method which uses TCA precipitation (6) and suggested that other compounds possessing a sulfhydryl group would have the same effect. When assayed by the method which uses ether extraction (5), recovery was not inhibited by cysteine. Because CoA contains a cysteamine moiety, and because stearoyl CoA might be expected to release CoA when incubated with microsomes, it was considered possible that the apparent inhibition of aniline p-hydroxylase activity by stearoyl CoA observed by Montgomery and Cinti (2) may in fact have been due to the low recovery of p-aminophenol that occurs when the TCA method is used. The data presented in Table 2 show this to be the case. In these studies hepatic microsomes were incubated with p-aminophenol for 0, 10 or 20 min with or without stearoyl CoA, oleoyl CoA or Na stearate. At the 0 min incubation time, recovery of p-aminophenol was lowered only when CoA was added and when the assay procedure used TCA. Because of the four compounds studied, only CoA possesses a sulfhydryl function, and because CoA did not affect recovery of p-

TABLE 2

Recovery of p-aminophenol and formaldehyde from incubation mixtures

The incubation medium was the same as that described in Table 1 except that in the p-aminophenol recovery studies, the mixture contained 1.0 rather than 0.6 mg of microsomal protein/ml and semicarbazide was omitted. Amounts of p-aminophenol (50 nmole) or HCHO (300 nmole) similar to those which would have been formed during incubation of aniline or ethylmorphine for 20 or 10 min, respectively, were added to 3.0 and 5.0 ml of incubation mixture, respectively, samples were removed for analysis after incubation at 37° for 0, 10 or 20 min. The values for the recovery of p-aminophenol are the means of 3 experiments; values for the recovery of HCHO are the means of 2 experiments.

Additions (50 μM)	% recovery of p-aminophenol				% recovery of HCHO	
	TCA Precipitation method (6)		Ether Extraction method (5)		HCHO method (3)	
	0 min	20 min	0 min	20 min	0 min	10 min
Stearoyl CoA	98	55	99	86	100	99
Oleoyl CoA	103	52	95	86	100	101
COA	60	63	107	100	99	101
Stearate	102	99	107	95	99	99

aminophenol when the ether extraction procedure was employed, it is concluded that recovery of p-aminophenol is lowered by CoA in the same manner that it is lowered by cysteine. At the 20 min incubation time, recovery of p-aminophenol was lowered by stearoyl CoA, oleoyl CoA and CoA when the TCA procedure was used. This is interpreted to mean that when stearoyl CoA and oleoyl CoA are incubated with microsomes, CoA is released, and this interferes with the recovery of p-aminophenol. When incubated with microsomes for 20 min, stearoyl CoA and oleoyl CoA retarded recovery of p-aminophenol by 16% (ether extraction method). The reason for this is not known. This loss was taken into account in subsequent assays of aniline p-hydroxylase activity, all of which used the ether extraction procedure.

As anticipated, none of the four compounds had an effect on the recovery of HCHO.

Effects of stearoyl CoA, oleoyl CoA, CoA and stearate on microsomal aniline p-hydroxylation, ethylmorphine N-demethylation and lipid peroxidation. The effects of stearoyl CoA, oleoyl CoA, CoA and stearate on aniline p-hydroxylation and ethylmorphine N-demethylation are shown in Table III. Because it seemed possible that stearoyl and oleoyl CoA might serve indirectly as substrates for the lipid peroxidation reaction, and because lipid peroxidases are known to depress monooxygenase activity (15–17), the effect of these compounds on lipid peroxidation was studied. The results are given in Table III. These studies used phosphate buffer, which contains enough iron to support lipid peroxidation at a relatively rapid rate or tris buffer, which does not (19). EDTA, which prevents lipid peroxidation by chelating iron (20), was omitted in appropriate cases. Because lipid peroxidation depresses monooxygenase activity by destroying cytochrome P-450 (19), rates of ethylmorphine or aniline metabolism obtained by the determination of the amount of product present in the medium at the end of the incubation period do not represent true initial velocities under conditions where high rates of lipid peroxidation were observed.

The data in Table 3 are summarized as follows: a) the rate of microsomal lipid peroxidation was enhanced by stearoyl and oleoyl CoA, but only when lipid peroxidation occurred at a relatively rapid rate in the absence of these compounds, i.e., when phosphate buffer was used and EDTA was omitted; b) CoA and stearate did not affect the rate of lipid peroxidation regardless of which buffer was used or whether or not EDTA was present; c) aniline p-hydroxylation was inhibited by stearoyl and oleoyl CoA (24 and 23%, respectively), but only when phosphate buffer was used in the absence of EDTA, i.e., only when the rate of lipid peroxidation was appreciable; d) CoA and stearate did not affect the rate of aniline p-hydroxylation appreciably under any of the conditions of this study; e) stearoyl CoA and oleoyl CoA inhibited ethylmorphine N-demethylation even when lipid peroxidation was minimal, but inhibition was about doubled when the rate of lipid peroxidation was elevated; f) stearate had a small inhibitory effect on the rate of ethylmorphine N-demethylation; g) when phosphate buffer was used and EDTA was omitted, lipid peroxidation occurred at a much lower rate when aniline was present than when ethylmorphine was present, which agrees with Gram and associates (21), who observed an inhibitory effect of aniline on lipid peroxidation. However, aniline did not lessen the enhancing effect of stearoyl or oleoyl CoA on lipid peroxidation. The following conclusions are derived from these observations: a) when a medium was used which is capable of supporting lipid peroxidation (phosphate buffer without EDTA), stearoyl and oleoyl CoA lowered the rate of aniline p-hydroxylation by about 25% and the rate of ethylmorphine N-demethylation by about 60%; all of the loss of aniline p-hydroxylation and about half of the loss of ethylmorphine N-demethylation would appear to be due to the effects of lipid peroxidation; b) when a medium was used which is not capable of supporting lipid peroxidation (Tris, Tris + EDTA, or phosphate + EDTA), stearoyl or oleoyl CoA did not cause a loss of aniline p-hydroxylase activity, but caused about a 35% inhibition of ethylmorphine N-demethylase activity; this fatty acyl CoA-induced loss of demethylase activity is due to an action other than

TABLE 3

Effects of phosphate (P) buffer, Tris (T) buffer and EDTA on the inhibitory effects of stearoyl CoA, oleoyl CoA, CoA and stearate on ethylmorphine (EM) N-demethylase, aniline p-hydroxylase, and lipid peroxidase activities of hepatic microsomes

The incubation medium was the same as that described in Table 1 except that when aniline was the substrate, the mixture contained 1.0 rather than 0.6 mg of microsomal protein/ml and semicarbazide was omitted. When aniline (0.25 mM) was the substrate, mixtures were incubated for 20 min (37°) with a 75 μM concentration of the added compounds and then analyzed for p-aminophenol and malondialdehyde; when EM (2 mM) was the substrate, the mixture was incubated for 10 min (37°) with a 50 μM concentration of the added compound and then analyzed for HCHO and malondialdehyde. Values are the mean ± SE of 3 experiments. Values in parentheses are percentages of corresponding controls (None).

Additions	Buffer	EDTA (50 μM)	Aniline Hydroxylation[a]	EM N-Demethylation[a]	Lipid peroxidation	
					+ Aniline[b]	+ EM[c]
None	P	−	.69 ± .01 (100)	4.65 ± .56 (100)	1.41 ± .32	8.65 ± 1.41
	P	+	.72 ± 00 (100)	6.00 ± .24 (100)	.71 ± .06	2.82 ± .51
	T	−		5.17 ± .24 (100)		1.08 ± .26
	T	+		5.12 ± .14 (100)		.96 ± .32
Stearoyl CoA	P	−	.56 ± .05 (76)	1.45 ± .39 (31)	16.73 ± 2.69	13.58 ± 1.92
	P	+	.69 ± .03 (98)	3.49 ± .17 (58)	0.64 ± 0.13	2.50 ± 0.51
	T	−		3.22 ± .17 (62)		1.22 ± .32
	T	+		3.48 ± .07 (68)		.83 ± .26
Oleoyl CoA	P	−	.56 ± .04 (77)	1.60 ± .34 (34)	1.59 ± .24	13.01 ± 1.47
	P	+	.72 ± .01 (101)	3.53 ± .21 (59)	.57 ± .13	2.50 ± .57
	T	−		3.20 ± .19 (62)		1.28 ± .38
	T	+		3.37 ± .15 (66)		.83 ± .32
CoA	P	−	.74 ± 00 (107)	4.57 ± .67 (98)	1.67 ± .58	8.40 ± 1.09
	P	+	.79 ± .01 (110)	5.90 ± .15 (98)	.64 ± .02	1.85 ± .03
	T	−		5.02 ± .23 (97)		1.22 ± .06
	T	+		5.28 ± .13 (103)		1.09 ± .38
Na Stearate	P	−	.64 ± .01 (92)	4.03 ± .55 (87)	1.73 ± .32	8.33 ± 1.35
	P	+	.65 ± .01 (91)	4.91 ± .08 (82)	.71 ± .06	1.73 ± .58
	T	−		3.67 ± .11 (71)		1.15 ± .26
	T	+		4.24 ± .16 (83)		.96 ± .32

[a] nmoles of p-aminophenol formed/mg of microsomal protein/min.

[b] malondialdehyde formation: nmoles/mg of microsomal protein observed after 10 min of incubation.

[c] malondialdehyde formation: nmoles/mg of microsomal protein observed after 20 min + 2.

the stimulation of lipid peroxidation.

Effect of 2′, 2′5′, 3′5′ and 5′ ADP on ethylmorphine N-demethylase activity. Rat hepatic microsomes contain nucleotide pyrophosphatase (22). The possibility therefore exists that microsomes could produce 3′5′ ADP from stearoyl CoA. Because 2′ ADP (22) and 2′5′ ADP[1] inhibit hepatic monooxygenase activity (22) it seemed possible that 3′5′ ADP might also have a similar effect. That CoA does not inhibit demethylase activity (Table 2), although it could also yield 3′5′ ADP, does not exclude the possibility that inhibitory amounts of 3′5′ ADP might be formed from stearoyl CoA because CoA is water soluble and would not be expected to penetrate the membrane as readily as stearoyl CoA. No inhibitory effect of 2′, 2′5′ or 3′5′ ADP (50 and 150 μM) on the N-demethylase activity of microsomes in the presence of 50 μM EDTA was observed. 5′ AMP (2 mM), which inhibits nucleotide pyrophosphatase almost completely, did not reverse the inhibitory effect of stearoyl CoA on demethylase activity. EDTA, at a concentration high enough to inhibit nucleotide pyro-

[1] E. Jeffery and G. J. Mannering, unpublished observations.

TABLE 4

Effect of oleic acid on microsomal ethylmorphine (EM) N-demethylase activity

The incubation medium was the same as that described in Table 1. Values are the mean ± SE of 3 experiments.

Concentration of oleic acid (μM)	EDTA	EM N-demethylation[a]	Lipid peroxidation[b]
0	−	6.3 ± .49 (100)	6.02 ± .77
	+	7.1 ± .40 (100)	1.54 ± .26
5	−	6.2 ± .49 (98)	6.60 ± .90
	+	7.3 ± .33 (103)	1.73 ± .19
10	−	6.2 ± .49 (98)	6.67 ± .51
	+	7.0 ± .41 (99)	1.61 ± .13
50	−	5.1 ± .25 (82)	8.40 ± .15
	+	6.8 ± .22 (97)	1.80 ± .32

[a] nmoles of HCHO formed/mg of microsomal protein/min.

[b] malondialdehyde formation: nmoles/mg of microsomal protein observed after 10 min of incubation.

phosphatase (200 μM), also failed to reverse the inhibition of demethylase activity by stearoyl CoA.

Effect of oleic acid on ethylmorphine N-demethylase activity. The possibility was considered that oleic acid derived from stearate released from stearoyl CoA might account for the observed inhibition of demethylase by stearoyl CoA. In Table 3 it can be seen that stearate inhibited ethylmorphine N-demethylase activity by about 15% and that the same degree of inhibition occurred regardless of whether lipid peroxidation occurred at a rapid (8.33 nmoles/mg/10 min) or a slow (0.96 nmoles/mg/10 min) rate. In Table 4 it can be seen that oleic acid did not inhibit demethylase activity until a concentration of 50 μM was reached, at which level the rate was decreased by 18%. This was also the concentration of oleic acid which caused an increase in lipid peroxidation. EDTA lowered lipid peroxidation and prevented the inhibitory effect of oleic acid on demethylase activity. It is concluded that the slight inhibitory effect of oleic acid on ethylmorphine N-demethylase is due to lipid peroxidation induced by oleic acid, whereas the slight inhibition caused by stearate is not.

Effect of stearoyl CoA on the generation of NADPH from $NADP^+$. Because stearoyl CoA is known to inhibit glucose-6 phosphate dehydrogenase (24), the NADPH-generating enzyme used in these studies, the possibility was considered that stearoyl CoA might produce its inhibitory effect on ethylmorphine N-demethylase activity by causing a rate limitation of NADPH. NADPH and $NADP^+$ concentrations of the complete medium containing ethylmorphine and 50 μM EDTA with and without the addition of 50 μM stearoyl CoA were determined at 0 and 10 min incubation periods. About half of the nucleotide disappeared during incubation, but the ratio of NADPH to $NADP^+$ was about 20 at both the beginning and the end of the incubation. At the end of the incubation period the concentration of NADPH in the medium containing stearoyl CoA was about the same as that found in the medium that did not contain stearoyl CoA (0.24 vs. 0.22 mM); this is greatly in excess of that required to saturate the system (25). Very similar results were obtained in the absence or presence of stearoyl CoA.

Effects of stearoyl and oleoyl CoA on NADPH-cytochrome c and NADPH-cytochrome P-450 reductases. Stearoyl or oleoyl CoA (50 μM) had no inhibitory effect on NADPH-cytochrome c reductase (data

TABLE 5

Effects of stearoyl CoA and oleoyl CoA on microsomal NADPH-cytochrome P-450 reductase activity

NADPH-cytochrome P-450 reductase activity was determined by the method of Gigon *et al.* (8) using 1.0 mg of microsomal protein/ml. Values are the mean ± SE of 3 experiments.

Additions	Reductase activity[a]	Δ Reductase activity[b]
None	10.9 ± 0.3	—
Stearoyl CoA (50 μM)	10.7 ± 0.8	−0.2
Ethylmorphine (EM) (2 mM)	14.6 ± 0.3[a]	3.7
Stearoyl CoA + EM	11.2 ± 1.0	0.5
None	10.9 ± 0.3	—
Oleoyl CoA (50 μM)	10.7 ± 0.4	−0.2
EM (2 mM)	14.6 ± 0.3[a]	3.7
Oleoyl CoA + EM	10.5 ± 0.2	−0.4

[a] nmoles P-450 reduced/mg of microsomal protein/min.

[b] reductase activity in the presence of added compounds minus reductase in the absence of these compounds.

not shown). In Table 5 it can be seen that in the absence of ethylmorphine, 50 μM stearoyl or oleoyl CoA had no inhibitory effect on NADPH-cytochrome P-450 reductase activity. However, the stimulatory effect of ethylmorphine on NADPH-cytochrome P-450 reductase activity was not observed when either fatty acyl CoA was present.

Effect of stearoyl CoA on the type I binding spectrum of ethylmorphine. The substrate activation of cytochrome P-450, which can be visualized as a type I binding spectrum, is thought to initiate monooxygenase activity. Because the type I binding spectrum appears to depend in part on the association of the cytochrome P-450 with membrane components, any disruption of the membrane by stearoyl CoA might be expected to produce a decrease in the magnitude of the type I spectrum of ethylmorphine. In Table 6 it can be seen that at the concentrations of microsomes (0.6 mg/ml), stearoyl CoA (50 μM) and ethylmorphine (2 mM) used in most of the preceding experiments, the type I spectrum produced by ethylmorphine was decreased by about 15%. A 27% decrease was observed when the concentrations of microsomes and stearoyl CoA were doubled. Denk and associates (26) produced greater losses of type I binding with synthetic anionic and nonionic detergents. Stearoyl CoA (50 or 100 μM) produced no spectrum of its own when added to microsomes.

Kinetics of the inhibition of ethylmorphine N-demethylase activity by stearoyl CoA. Fig. 1 shows double reciprocal plots of the inhibitory effects of several concentrations of stearoyl CoA on the microsomal N-demethylation of ethylmorphine. The figure shows that stearoyl CoA does not inhibit demethylase activity by competing as a substrate.

DISCUSSION

Fatty acyl CoA inhibits NADPH-supported cytochrome P-450-dependent monooxygenase systems (1). In theory, this could occur in several ways: a) Electrons from NADPH might be diverted from cytochrome P-450 via cytochrome b_5 to the fatty acyl CoA desaturase system. This is not the case because cyanide, in concentrations which are known to inhibit the desaturase system almost completely, did not reverse fatty acyl CoA inhibition of ethyl-

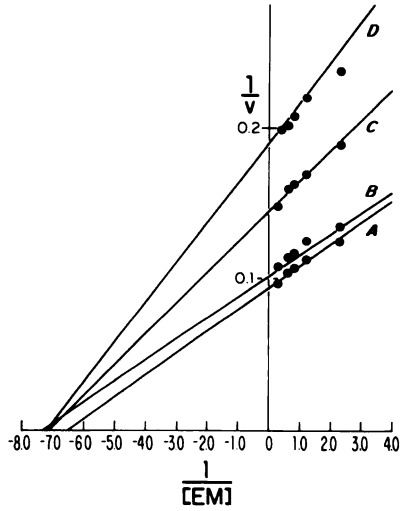

FIG. 1. *Inhibition of ethylmorphine N-demethylase activity by 0 (A), 10 (B), 50 (C) and 100 (D) μM stearoyl CoA*

Apparent Km values (mM) and Vmax values (nmol/mg of protein/min) for A, B, C and D, respectively, are 0.15 ± 0.04 and 10.59 ± 0.62; 0.14 ± 0.04 and 9.55 ± 0.40; 0.14 ± 0.03 and 6.92 ± 0.12; 0.14 ± 0.05 and 5.21 ± 0.04.

TABLE 6

Effect of stearoyl CoA on the type I binding spectrum of ethylmorphine

Values are Δ $OD_{385-420\ nm}$ ± SE; N = 3. Values in parentheses are percentages of the values obtained in the absence of stearoyl CoA.

Concentration of microsomes (mg of protein/ml)	Concentration of stearoyl CoA		
	0	50 μM	100 μM
0.6	.0086 ± .0003	.0070 ± .0005 (82)[a]	.0062 ± .0006 (72)[b]
1.2	.0207 ± .0007	.0172 ± .0012 (83)[a]	.0154 ± .0006 (74)[b]

[a or b] Significantly different ($p < .1$ or .05, respectively) from values obtained in the absence of stearoyl CoA.

morphine N-demethylase (1). b) Fatty acyl CoA or the fatty acids derived from fatty acyl CoA might be hydroxylated by cytochrome P-450-dependent monooxygenase systems, thereby serving as substrate inhibitors of ethylmorphine N-demethylase. This is not the case, at least to an appreciable degree, because stearate had only a small inhibitory effect (Table 3). Oleic acid had a similar effect, but this did not occur when lipid peroxidation was prevented with EDTA (Table 4). Moreover, the kinetics of fatty acyl CoA inhibition of demethylase activity is not compatible with substrate inhibition (Fig. 1). c) 2′ and 2′5′ ADP inhibit microsomal monooxygenase activity (23). 3′5′ ADP produced through the action of microsomal pyrophosphatase might also inhibit monooxygenase systems; this proved not to be the case. d) Fatty acyl CoA might stimulate lipid peroxidation, which is known to depress monooxygenase activity (15–17). This could occur because the fatty acid is desaturated and then peroxidated in the microsomes, or a detergent action of fatty acyl CoA could make endogenous unsaturated fatty acids more available for peroxidation. Fatty acyl CoA stimulated lipid peroxidation. When lipid peroxidation was prevented, about half of the inhibition of demethylation was prevented (Table 3). e) Fatty acyl CoA has the hydrophilic and hydrophobic components of a detergent. Many of the properties of the membrane bound cytochrome P-450-dependent systems are thought to depend in part on their intimate association with membrane components. Disassociation of these highly complexed systems by detergent action might be expected to decrease the activities of certain of these monooxygenase systems. The kinetics of the inhibition of ethylmorphine N-demethylase activity by stearoyl CoA (Fig. 1) is compatible with a detergent effect. The observed loss of Δ NADPH-cytochrome P-450 reductase activity (Table 5) without a loss of NADPH-cytochrome c reductase activity might also be interpreted to reflect a detergent effect on the association of the substrate with cytochrome P-450.

These studies show that half of the inhibitory action of stearoyl CoA is due to stimulation of lipid peroxidation. Part or all of this may be initiated by detergent action. The mechamism for the remaining half of the inhibition might also be explained by a detergent-induced disturbance of the enzyme-substrate complex. If the membrane structure contributes to the activity of cytochrome P-450 systems and this structure is disturbed by detergent action, the overall effect would be equivalent to a loss of enzyme, i.e., the loss of activity would not be accompanied by a change in K_m, which is the case (Fig. 1). The loss of type I binding (Table 6) might also be influenced by a detergent action of stearoyl CoA. The hydroxylation of aniline, a type II compound, was not inhibited by fatty acyl CoA, which might suggest that if a detergent effect is involved, it is more specifically involved with the type I binding site than with other features of the monooxygenase system. In this connection, it is worth noting that type I compounds stimulate NADPH-cytochrome P-450 reductase activity, but type II compounds do not (9) and that stearoyl CoA prevented the stimulation of NADPH-cytochrome P-450 reductase by ethylmorphine (Table 5). This might explain why stearoyl CoA inhibited the N-demethylation of ethylmorphine, a type I compound, but not the hydroxylation of aniline, a type II compound.

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