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Pyridine Nucleotide-Dependent Electron Transport in Kidney Cortex Microsomes: Interaction with Desaturase and Other Microsomal Mixed-Function Oxidases

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SUMMARY

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Rat kidney cortex microsomes contain both cytochrome b_5 and its flavoprotein reductase, yet do not catalyze fatty acyl-CoA desaturation. This inactivity was unaltered by feeding a high-carbohydrate diet. However, supplementation of liver or lung microsomes with kidney microsomes produced a significant increase in desaturase activity. Kidney microsomes had no effect on liver *N*-demethylation or aromatic *p*-hydroxylation and only an additive effect on aryl hydrocarbon hydroxylase activity, suggesting specificity for the kidney-dependent synergism of desaturase. Although stearoyl-CoA is not desaturated by kidney microsomes, it did inhibit both kidney aryl hydrocarbon hydroxylase activity and NADH-cytochrome *c* reductase activity, which was restored to the control level by cyanide; stearic acid had no effect on reductase activity. These results suggest the presence of a desaturase electron transport system in kidney which may lack a factor(s) normally present in liver and lung.

INTRODUCTION

Like liver and lung, the kidney cortex microsomal fraction possesses two cytochrome-linked electron transport chains which utilize reducing equivalents in the forms of NADH and NADPH. One of these chains, which contains the flavoprotein NADPH-cytochrome P-450 reductase and cytochrome P-450, is involved in catalyzing numerous oxidative reactions (1). However in the kidney, only ω and ω-1 hydroxylation of fatty acids (2, 3) and the hydroxylation of benzopyrene (4, 5) are catalyzed to a significant extent by this enzyme system.

The second electron transport chain contains at least two components, the hemoprotein cytochrome b_5 and its NADH-dependent flavoprotein reductase. Until recently, the physiological role of this electron transport system in liver and lung has been unclear. Evidence for liver, based on NADH synergism with NADPH-dependent drug oxidations (6), on the partial

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reoxidation of reduced cytochrome b_5 in the presence of cytochrome P-450 drug substrate (7), and on anti-cytochrome b_5 antibody studies (8–10), suggests a transfer of reducing equivalents from NADH to the ternary cytochrome P-450 complex via cytochrome b_5.

Other investigations have provided strong evidence indicating that this NADH-dependent microsomal electron transport chain catalyzes the oxidative Δ^9 desaturation of fatty acyl-CoAs to their corresponding monoenoic acid derivatives (11–15). In liver, adipose tissue, and lung, this enzyme system also contains a third component, which, because of its sensitivity to cyanide, has been termed the cyanide-sensitive factor (desaturase) (11, 16). In studies designed to elucidate the functional relationship among these components, no clear correlation has been observed between tissue cytochrome b_4 content and desaturase activity. For example, although adipose tissue contains about one-fifth the cytochrome b_4 present in liver, adipose desaturase activity is more than twice that of liver (17). Lung, which possesses about one-half the liver desaturase activity (16) in the normal rat, has only one-tenth the amount of liver cytochrome b_4. Moreover, although the kidney contains to one-fourth one-third the amount of cytochrome b_4 (1, 17, 18) present in liver, there is no measurable desaturase activity (17, 19).

Furthermore, the desaturase system in both liver and lung is highly inducible by dietary manipulations. Feeding a high-carbohydrate diet by various schedules may result in as much as a 10–15-fold increase in liver desaturase activity (20, 21) and a 2–3-fold increase in lung activity (16). However, there is no apparent relationship between cytochrome b_4 content and the induction phenomenon, since there is no enhancement of microsomal cytochrome b_4 concentration in these tissues (16, 20); indeed, it has been reported that hepatic cytochrome b_4 levels have decreased following this induction (22). Although under these dietary conditions the liver and lung desaturase activities are highly induced, the kidney microsomal system still fails to exhibit desaturase activity toward stearoyl-CoA (19), the preferred substrate of the liver and lung Δ^9 desaturase systems (16, 23).

In light of our previous observations of significant NADH-cytochrome b_4 reductase activity and cytochrome b_4 content in kidney cortex microsomes (19), the present study was designed to investigate the absence of stearoyl-CoA desaturase activity in these preparations. This study reports that kidney cortex microsomes contain an electron transport system which is sensitive to the presence of stearoyl-CoA and cyanide and does not desaturate stearoyl-CoA to oleoyl-CoA, but is capable of significantly stimulating functional desaturase systems in other tissues.

METHODS

Preparation of microsomes. Male Sprague-Dawley rats, 175–275 g, were given access ad libitum to Purina rat chow or a high-carbohydrate diet ("fat-free" test diet, Nutritional Biochemicals) and tap water for 4 days prior to death. 3-Methylcholanthrene (40 mg/kg in corn oil, intraperitoneally) was administered once daily for 2 days prior to death. All animals were killed by decapitation between 8:00 and 9:00 a.m. The organs to be studied were removed and homogenized in a motor-driven Teflon-glass homogenizer with 0.10–0.15-mm clearance in 3 volumes of 0.02 M Tris–0.15 M KCl buffer, pH 7.4, or 0.25 M sucrose. For preparation of lung microsomes, prior to homogenization the lungs were washed free of adhering blood and then finely minced in buffer, and the mince was washed with buffer until the wash solution was clear. For preparation of kidney cortex microsomes, the cortex was isolated from the medulla prior to homogenization. Liver and lung homogenates were then centrifuged at 9000 × g for 15 min; kidney was centrifuged at 15,000 × g for 15 min. The supernatant was centrifuged at 165,000 × g for 40 min, and the resultant pellet was resuspended in Tris-KCl buffer and resedimented at 165,000 × g for 40 min. The microsomal pellet was then resuspended in Tris-KCl. Microsomal protein was de-

termined by the method of Sutherland *et al.* (24) or Lowry *et al.* (25).

Kidney microsomes prepared with Tris-KCl buffer contained virtually no hemoglobin (as shown by the lack of a CO-induced absorption peak at 420 nm), a hemoprotein which might affect spectral determinations of microsomal cytochromes. However, the mitochondrial contamination, as measured by succinate-cytochrome *c* reductase activity, was approximately 5% of the activity observed in a mitochondrial fraction. On the other hand, homogenization of kidney cortex in 0.25 M sucrose reduced the mitochondrial contamination to approximately 1% but resulted in significant hemoglobin contamination in the final microsomal suspension. The enhanced liver and lung desaturase activities were the same for either kidney microsomal preparation. Since mitochondrial contamination could easily alter electron flow through the microsomal electron transport chains, sucrose-prepared kidney microsomes were employed for measuring electron transport components. Liver and lung microsomes were prepared only with Tris-KCl.

Materials. 3-Methylcholanthrene was obtained from Sigma Chemical Company; stearic acid, from Applied Science Laboratories; benzo[*a*]pyrene, from Eastman Kodak; aminopyrine, from Aldrich Chemical Company; and ethylmorphine hydrochloride, from Mallinckrodt. All other chemicals were of the highest commercial grade available.

Assays of enzymatic activities. Desaturation of [1-^{14}C]stearoyl-CoA or [1-^{14}C]-palmitoyl-CoA was determined as previously described for lung (16), kidney (19), and liver (21) microsomes. Briefly, 0.1 ml of the microsomal suspension, containing 1.0 mg of microsomal protein, was added to the the incubation mixture, which contained 1 mM NADH and either 70 μM [1-^{14}C]stearoyl-CoA for liver experiments or 35 μM [1-^{14}C]stearoyl-CoA for kidney and lung experiments, in a final volume of 0.50 ml of 0.1 M Tris-HCl buffer, pH 7.25. [1-^{14}C]Stearoyl-CoA (New England Nuclear, lot 678-279; specific activity, 51.8 mCi/mmole), 0.2 mg (0.010 mCi), was added to 10 mg of unlabeled stearoyl-CoA (Sigma) in Tris-HCl, pH 7.25, for use in the incubation mixtures. [1-^{14}C]Palmitoyl-CoA (New England Nuclear, lot 748-221; specific activity, 57.8 mCi/mmole) was prepared similarly with unlabeled palmitoyl-CoA (Sigma) for use with kidney microsomes. All incubations were performed in duplicate for 4 min (liver) or 6 min (lung or kidney) at 37° in a shaking water bath. The reaction was stopped by the addition of 1.0 ml of 10% KOH in methanol, followed by saponification at 80° for 30 min. The mixture was then acidified with 2.0 ml of 4 N HCl, and the fatty acids were extracted with 30 ml of petroleum ether and converted to methyl esters with 3 ml of 14% boron trifluoride in methanol (26). The methyl esters were extracted into petroleum ether and separated by thin-layer chromatography on silica gel GF plates (Analtech) containing 10% $AgNO_3$ developed in ether–hexane (1:9, v/v). The spots were identified under ultraviolet light after spraying with 0.05% Rhodamine B (in methanol) and compared with authentic standards. The spots were scraped and counted in a toluene scintillator (4 g of 2,5-diphenyloxazole and 0.25 g of 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene per liter of toluene) in a Beckman LC-100 scintillation counter. Desaturation activity was determined by dividing the radioactivity found in the monounsaturated ester by the sum of the radioactivities in both the saturated and unsaturated esters; the ratio was then converted to nanomoles of oleic formed per minute per milligram of microsomal protein. Background activity for assay was determined by addition of the microsomes after the KOH.

Hydroxylation of benzo[*a*]pyrene was measured according to Gnosspelius *et al.* (27) with slight modification. Benzo[*a*]-pyrene was dissolved in acetone rather than ethanol and added first to the incubation flask; either the evaporation of acetone or its presence in the incubation medium resulted in the same benzo[*a*]pyrene hydroxylase activity. The concentration of benzo[*a*]pyrene in each flask was 75 μM. The incubation mixture contained 50

μmoles of Tris-Cl buffer (pH 7.5), 5 μmoles of $MgCl_2$, 1.0 μmole of NADPH, and 0.5 mg of microsomal protein in a total volume of 1.0 ml. Incubations were conducted for 20 min at 37° in air. The NADPH-generating system consisted of 1.0 mM isocitrate, 5.0 mM $NADP^+$, and isocitrate dehydrogenase (15 μg/ml, Sigma type IV). All hydroxylation reactions were initiated by the addition of microsomes, and all determinations were performed in triplicate. The amount of hydroxylated substrate formed during the incubation was estimated fluorometrically, using 3-hydroxybenzo[*a*]pyrene as a standard.

Aminopyrine and ethylmorphine *N*-demethylase activities were determined by estimation of formaldehyde production with Nash's reagent (28). Aminopyrine and ethylmorphine hydrochloride were used as substrates at 5.0 mM and 2.0 mM, respectively. The NADPH-generating system consisted of $NADP^+$ (0.4 mM), glucose-6-P (4 mM), and glucose-6-P dehydrogenase (2 enzyme units) in a final volume of 3.0 ml. Reaction mixtures were incubated under air at 37° for 15 min; the reaction rates were linear throughout this period.

Aromatic *p*-hydroxylation of aniline was determined essentially as described by Schenkman *et al.* (29), by measuring *p*-aminophenol formation. The hydroxylation reaction, supported by the same NADPH-generating system in a 3-ml volume as described for *N*-demethylase, was terminated after 20 min at 37° by addition of 1.5 ml of 20% trichloracetic acid. Following centrifugal separation of the precipitated protein, 1 ml of 0.5 M NaOH containing 1% phenol was added to 1.0 ml of the supernatant. After mixing, 1 ml of 1 M Na_2CO_3 was added, and the mixture was incubated at room temperature for 20 min. *p*-Aminophenol formation was determined at 630 nm.

NADH–cytochrome b_5 reductase activity, using ferricyanide as the electron acceptor, was measured by the procedure of Strittmatter (30) with an Aminco DW-2 spectrophotometer. NADH–cytochrome *c* reductase activity was estimated as described by Dallner (31), and succinate–cytochrome *c* reductase was determined by the method of Sottocasa *et al.* (32) by following the reduction of cytochrome *c* at 550 nm. The assay mixture contained 0.1 mM NADH or 3 mM succinate, 0.1 mM cytochrome *c*, 0.3 mM KCN, and 50 mM phosphate buffer, pH 7.5. The reaction was started by the addition of the substrate.

Statistics. Statistical analysis of the effect of kidney microsomes on both liver and lung desaturase activities was performed using the paired *t*-test. This method is appropriate for determination of significance ($p < 0.05$) in a paired experimental design such as utilized here for liver or lung activity alone compared with the activity determined upon the addition of kidney microsomes.

RESULTS

Kidney cortex microsomes prepared from rats maintained on either the control or the fat-free diet failed to demonstrate any Δ^9 desaturase activity with either stearoyl-CoA or palmitoyl-CoA as substrate. This result was consistently observed whether NADH, NADPH, or an NADPH-generating system was utilized as the source of reducing equivalents. This observation was most surprising in view of (a) the observed cytochrome b_5 concentrations of 0.15 ± 0.02 and 0.12 ± 0.01 nmole/mg of microsomal protein for rats maintained on the control and fat-free diets, respectively (N = 4/group) and (b) the observed cytochrome b_5 reductase activities of 1.62 ± 0.05 and 1.50 ± 0.27 μmoles/min/mg of protein for the same two diets, respectively (N = 4/group). Furthermore, both the kidney cortex microsomal cytochrome b_5 content and cytochrome b_5 reductase activity were significantly greater than in lung microsomes, which possess significant desaturase activity (16); lung b_5 was 0.07 and 0.05 nmole/mg of protein and b_5 reductase was 1.43 and 1.48 nmoles/min/mg of protein for the normal and fat-free diets, respectively.

In order to determine whether kidney microsomes could be activated for desaturase activity, stearoyl-CoA desaturase activity was measured when these microsomes were in the presence of an intact system. Addition of kidney microsomes to

liver (Fig. 1) or lung (Fig. 2) microsomes resulted in more than a 2-fold stimulation of basal liver or lung desaturase activity. As expected, the fat-free diet markedly induced both the liver and lung activities, which were enhanced (20%) significantly ($p < 0.01$) further by the presence of kidney microsomes; dietary treatment did not affect the kidney-induced activity. It should be pointed out that, although the liver activity following the fat-free diet was increased further by only 20% upon addition of kidney microsomes, the absolute increase in activity was 0.95 nmole of oleate formed per minute per milligram of protein, compared with an increase of 0.46 nmole/min/mg for the control diet liver preparation.

In a separate experiment, the effect of increasing concentrations of kidney microsomes on liver desaturase activity was determined (Table 1). While the over-all activity was somewhat lower in this experiment, significant enhancement of total desaturase activity was observed with the lowest concentration (0.5 mg/0.5-ml incubation) of kidney microsomes added. This stimulation progressively increased with higher concentrations of kidney microsomes (Table 1). These concentrations of kidney microsomes had no detectable desaturase activity (less than 0.1 nmole/min/mg) when examined alone. The substitu-

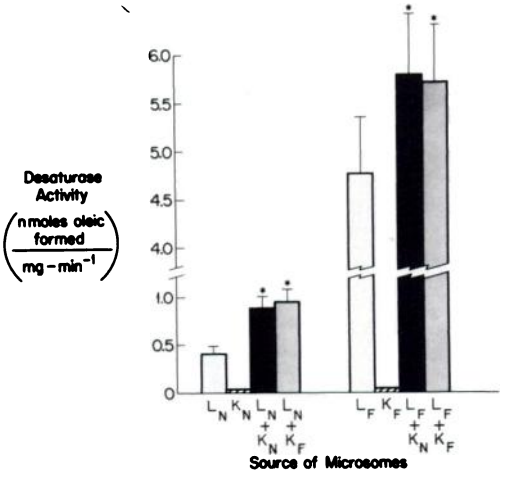

FIG. 1. *Stimulation of liver stearoyl-CoA desaturase activity by kidney microsomes*

L_N and K_N represent microsomes prepared from liver and kidney cortices from rats maintained on the control diet; L_F and K_F represent similar preparations from rats given the fat-free diet. N = six rats per dietary treatment. The cofactor was 1 mM NADH. Data are means ± standard errors.

* $p < 0.01$ by the paired t-test.

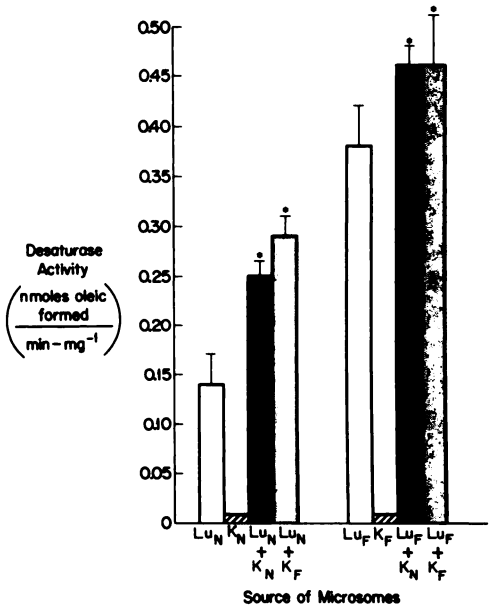

FIG. 2. *Stimulation of lung stearoyl-CoA desaturase activity by kidney microsomes*

Lu represents lung microsomes. Other details were the same as for Fig. 1.

* $p < 0.01$ by the paired t-test.

TABLE 1

Effect of increasing concentrations of kidney microsomes on liver microsomal fatty acid desaturase

Separate microsomes were prepared from three rats given the fat-free diet. Kidney microsomes were added to liver microsomes prepared from the same rat. The cofactor was 1 mM NADH, and the total incubation volume was 0.5 ml, as described in METHODS. Data are means ± standard errors for duplicate incubations.

Kidney microsomes added to 1.0 mg of liver microsomes	Total desaturase activity	Per cent of liver activity
mg	*nmoles/min/mg*	*%*
0	1.80 ± 0.33	100
0.5	2.47 ± 0.37	145 ± 11
1.0	2.90 ± 0.47	170 ± 13
2.0	3.39 ± 0.40	206 ± 25

tion of 1 mM NADPH for 1 mM NADH resulted in a 25–35% decrease in over-all desaturase activity; however, the stimulatory effect of the kidney microsomes persisted.

The stimulation of liver or lung desaturase activity was lost following addition of kidney microsomes which had been boiled for 5 min. No measurable desaturase activity was observed when boiled liver or lung microsomes were added to kidney microsomal preparations. This indicates that both systems (liver plus kidney, lung plus kidney) must be intact for expression of the simulatory effect. The kidney-stimulated activity was not a nonspecific protein interaction, since substitution of an equivalent concentration of bovine serum albumin (2 mg/ml) did not alter either liver or lung desaturase activity. Nor did the addition of bovine serum albumin to kidney microsomes result in any demonstrable activity.

Both the stearoyl-CoA desaturase activity and the observed stimulation with kidney microsomes were unaltered by several types of perturbations prior to incubation: (a) rapid freezing and thawing (15 times) of the liver or kidney microsomes, (b) extraction of liver and kidney microsomes with 100 mM butanol, (c) preparation of microsomes with the ionic medium of Catala *et al.* (33), which has been reported to remove a stimulatory factor from liver, or (d) addition of a 105,000 × *g* liver supernatant fraction to kidney microsomes.

Triton X-100 has been effective in unmasking several latent enzymatic activities [e.g., UDP-glucuronyltransferase (34), malate dehydrogenase (35), and glutamate dehydrogenase (35)]. Various concentrations up to 1% detergent were ineffective in unmasking any kidney desaturase activity. Indeed, for this particular enzyme system, Shimakata *et al.* (14) have reported that 1% Triton X-100 was significantly inhibitory.

In order to investigate the specificity of the stimulatory phenomenon, other microsomal mixed-function oxidases, which, like the desaturase, are active in liver but not in kidney, were examined. The liver microsomes actively catalyzed the *N*-demethylation of aminopyrine and ethylmorphine and also the *p*-hydroxylation of aniline; kidney microsomes were devoid of these activities (Table 2). The combined kidney and liver systems did not enhance the enzymatic activity above that of liver alone for any of the substrates (Table 2).

If the stimulatory effect is indeed specific for the desaturase system, determination of a mixed-function oxidase activity found in both liver and kidney should result in only an additive effect when the two microsomal systems are combined. Such was, in fact, observed for aryl hydrocarbon hydroxylase activity utilizing benzo[*a*]pyrene as the substrate (Table 3). This additive response was unaltered by induction of the hydroxylase with 3-methylcholanthrene (Table 3).

Correia and Mannering (6) have reported that the desaturase substrate, stearoyl-CoA, inhibited liver microsomal mixed-function oxidation. This inhibition apparently resulted from a diversion of electrons from the NADPH-dependent cytochrome P-450 pathway to the functional cytochrome b_5-dependent desaturase electron transport system. Confirmation of their observations with *N*-demethylations is presented in Fig. 3, where ethylmorphine and aminopyrine *N*-demethylases were slightly inhibited by 0.1 mM stearoyl-CoA and significantly inhibited by 0.5 mM stearoyl-CoA. Furthermore, a second mixed-function oxidation, *p*-hydroxylation

TABLE 2

Rat liver microsomal N-demethylation and hydroxylation in the presence and absence of rat kidney cortex microsomes

Data represent averages of duplicate incubations in two separate experiments. The cofactor was the NADPH-generating system.

Source of microsomes	Microsomal oxidations		
	Aminopyrine	Ethylmorphine	Aniline
	nmoles product/min/mg protein		
Liver (1 mg/ml)	11.7	20	0.16
Liver (1 mg/ml) + kidney (1 mg/ml)	12.6	18.2	0.19
Kidney (1 mg/ml)	<0.5	<0.4	0.008

of aniline, was almost completely inhibited (more than 90%) by 0.1 mM stearoyl-CoA (Fig. 3). The addition of 0.1 mM CN^- had no effect on the stearoyl-CoA-induced inhibition of aniline metabolism.

Since desaturase activity was not demonstrable in the kidney, one would expect that stearoyl-CoA would be ineffective in this tissue, unlike the liver, in altering electron flow from an NADPH-dependent oxidative reaction. To our surprise, low concentrations of stearoyl-CoA (0.1–0.4 mM) caused significant inhibition of aryl hydrocarbon hydroxylase activity in kidney cortex microsomes (Fig. 4); similar inhibition was also observed in kidney microsomes prepared from rats treated with 3-methylcholanthrene.

In view of this inhibition of aryl hydrocarbon hydroxylase activity, we examined the effect of stearoyl-CoA on components of the kidney microsomal NADH-dependent electron electron transport system. Although NADH-cytochrome b_5 reductase activity was unaffected by concentrations of stearoyl-CoA up to 0.4 mM, NADH-cytochrome *c* reductase activity was significantly decreased by as little as 0.1 mM stearoyl-CoA and almost totally inhibited by 0.4 mM stearoyl-CoA (Fig. 5). This inhibition was reversed by 0.15 mM cyanide, suggesting the presence of a factor which is sensitive to cyanide. In contrast to stearoyl-CoA, stearic acid, which is not a substrate for desaturase, had no effect on NADH-cytochrome *c* reductase activity (Fig. 5).

TABLE 3

Aryl hydrocarbon hydroxylase activity in microsomes of liver, kidney, and liver plus kidney from control and 3-methylcholanthrene-treated rats

Data are averages of triplicate incubations in two separate experiments. The cofactor was 1 mM NADPH.

Source of microsomes	Aryl hydrocarbon hydroxylase activity
	nmoles product/hr/mg protein
Control	
Liver (0.1 mg/ml)	34.5
Kidney (0.1 mg/ml)	6.4
Liver + kidney (each 0.1 mg/ml)	42.6
3-Methylcholanthrene-treated	
Liver (0.1 mg/ml)	113.5
Kidney (0.1 mg/ml)	36.0
Liver + kidney (each 0.1 mg/ml)	144.0

DISCUSSION

Our data indicate that kidney cortex microsomes contain two functional components of an NADH-dependent electron transport chain, i.e., cytochrome b_5 reductase and cytochrome b_5, and possibly a third component, which is suggested by the stearoyl-CoA sensitivity studies. These components do not catalyze stearoyl-CoA desaturation, but do stimulate

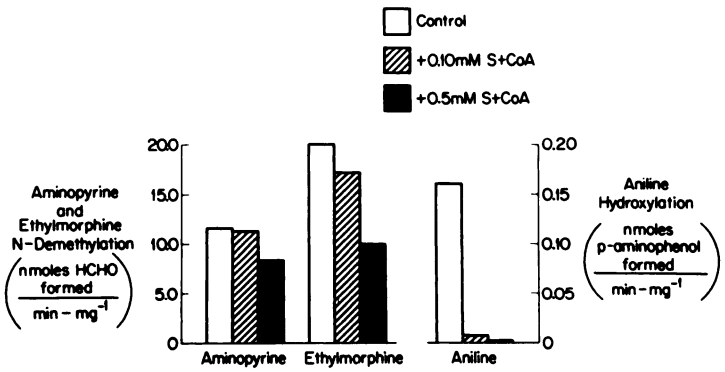

FIG. 3. *Effect of stearoyl-CoA (StCoA) on hepatic aminopyrine and ethylmorphine N-demethylation and analine p-hydroxylation*

Data are the averages of duplicate determinations for two separate experiments. The cofactor was the NADPH-generating system.

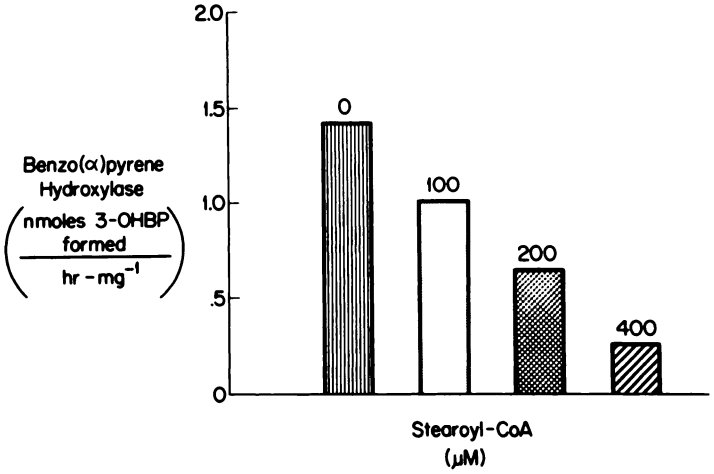

FIG. 4. *Effect of stearoyl-CoA on kidney benzo[a]pyrene hydroxylation*

Data are the averages of duplicate determinations for two separate experiments. The cofactor was 1 mM NADPH. 3-OHBP, 3-hydroxybenzo[a]pyrene.

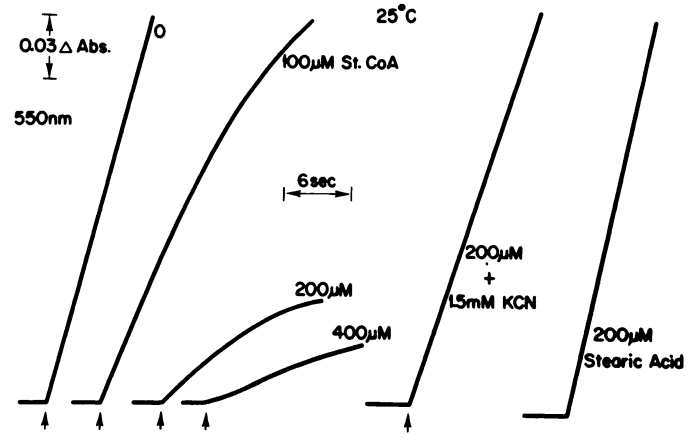

FIG. 5. *Effect of stearoyl-CoA (StCoA), cyanide, and stearic acid on kidney NADH-cytochrome c reductase activity*

The enzymatic activity was measured as described in METHODS. All reactions were initiated by addition of NADH (indicated by the arrows at bottom); 0.15 mM KCN alone had no effect on NADH-cytochrome *c* reductase activity.

liver or lung desaturase activity. This stimulation may be due to one of several mechanisms. Kidney cortex microsomes may lack the terminal component of the desaturase system, but do contain an NADH-cytochrome *c* reductase system which is fully active when the desaturase is supplied from another microsomal source. The 2-fold increase in desaturase activity found with kidney plus liver or lung microsomes cannot be explained simply by the addition of more cytochrome b_5 reductase and/or cytochrome b_5. The reductase activity in control rat liver microsomes is about 4–7 μmoles/min/mg of protein(30), which is four orders of magnitude greater than the stearoyl-CoA desaturase activity. Addition of another 1.6 μmoles/min/mg of reductase activity from kidney microsomes to a system already containing a 4000-fold excess could not explain the observed increase. Furthermore, only 0.15 nmole of cytochrome b_5 per milligram of kidney protein was added to the liver mi-

crosomal system, which contained 0.5–0.6 nmole of b_5 per milligram. Jansson and Schenkman (22) reported recently that fortifying rat liver microsomes with purified cytochrome b_5 enhanced desaturase activity in control rats, but only slightly; for example, the binding of 4–5 times more cytochrome b_5 (2.5 nmoles/mg) to control rat liver microsomes resulted in an increase in desaturase activity from about 0.3 nmole of oleate formed per minute per milligram of protein to 0.4 nmole/min/mg. Moreover, the fat-free diet caused a 10–20-fold increase in desaturase activity with a concomitant decrease in both cytochrome b_5 and its reductase (20); our observations were consistent with these results. Hence the stimulation cannot be explained by supplementation of the incubation mixture with these components.

The possibility that the stimulation was related to nonspecific protein stabilization of the desaturase, as reported for other systems (36 and 37), was excluded because neither an equivalent concentration of bovine serum albumin nor boiled microsomes produced the stimulatory effect. To the contrary, the stimulation was specific, since liver mixed-function oxidases, inactive in kidney, were not stimulated by the intact kidney microsomes. Furthermore, aryl hydrocarbon hydroxylase, active in both tissues, showed only an additive effect when these tissues were incubated together.

A third possibility may be the presence of a "repressor" substance in kidney microsomes, which is removed or rendered inactive upon interaction with liver or lung microsomes. This hypothesis is not unreasonable, since it has been reported recently that rat kidney homogenates contain an inhibitor of 25-hydroxy vitamin D_3 1-hydroxylase activity (38). However, in our system this appears unlikely, since prior treatment of kidney microsomes by several procedures designed to remove such a substance did not elicit any desaturase activity. Specifically, albumin, butanol extraction, preparation of microsomes in a medium of high ionic strength (33), freezing and thawing, and detergent treatment were uniformly ineffective.

Another possibility is the existence in kidney cortex microsomes of a factor which stimulates liver and lung desaturase. At present we have no definitive evidence to substantiate or reject this hypothesis, although initial experiments using butanol extraction and freezing-thawing procedures have failed to remove the stimulatory effect. If, indeed, this hypothesis is correct, the question remains as to the function of NADH–cytochrome b_5 reductase and cytochrome b_5 in rat kidney cortex microsomes.

Last, the inhibition of kidney microsomal aryl hydrocarbon hydroxylase activity by stearoyl-CoA suggests that this acyl-CoA must interact with some component of the kidney microsomal electron transport system. Furthermore, the observation that the stearoyl-CoA-mediated decrease in NADH–cytochrome *c* reductase activity is reversed by cyanide suggests the presence of a cyanide-sensitive factor (desaturase) in these microsomes. Hence the data suggest that the kidney desaturase requires activation by a factor absent from kidney microsomes but normally present in liver and lung. This implies that both liver and lung desaturase systems contain a fourth component, as yet unidentified, which is obligatory for desaturase activity. This possibility is not excluded by the reconstitution studies of Shimakata *et al.* (14) and Strittmatter *et al.* (39), since in their studies not all the components were highly purified. Investigations are continuing to elucidate further the characteristics of the kidney desaturase electron transport system.

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