Formation of Monoenoic Fatty Acids by Desaturation in Rat Brain Homogenate

Some Properties of the Enzyme System of 10-Day-Old Brain

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Summary

Desaturation of [1-14C]stearic acid, [1-14C]palmitic acid, and [1-14C]stearoyl-CoA was measured in homogenates of 10-day-old rat brain. Desaturation was maximal with stearoyl-CoA at pH 6.0 to 6.6. With free fatty acids, monoene formation was maximal at pH 7.2 to 7.6; stearic acid was more actively desaturated than palmitic acid. Activity with stearic acid or stearoyl-CoA was linear with protein concentration of 2 to 6 mg per ml and time to 10 min and was optimal at 37 to 45°C. At pH 7.4, fatty acid desaturation was not limited by the formation of fatty acyl-CoA derivatives when coenzyme A, Mg2+, and ATP were added. Rapid loss of activity in vitro prevented accurate subcellular location of the enzyme system. Oxygen and NAD+ or NADH were necessary for maximal activity. Diphosphonucleotides were more active than triphosphonucleotides, and oxidized were as effective as reduced forms. The monoene fraction from desaturation of the labeled stearic acid was 91% oleic acid (18:1(n-9)); desaturation of palmitic acid produced 22% palmitoleic acid (16:1(n-7)) and 72% cis-vaccenic acid (18:1(n-7)). At concentrations approximating normal tissue levels, these monoenes did not inhibit desaturation.

Enzymatic desaturation of saturated fatty acids to form mono-unsaturated fatty acids (monoenes) is known to occur in various aerobic organisms, including yeast (1, 2) and bacteria (3), plants (4–6), and several animal species (6–13). Studies with mammals have concerned mainly the desaturation of stearic acid and palmitic acid in whole homogenates or subcellular fractions of liver, mammary gland, small intestine, and adipose tissue.

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‡ Research Associate of the Medical Research Council of Canada.

Experimental Procedure

Materials—[1-14C]Stearic acid (48.4 mCi per mmole) and [1-14C]palmitic acid (50 mCi per mmole) were obtained from Amersham-Searle Corp., Don Mills, Ont., Canada, and [1-14C]-stearoyl coenzyme A (11.2 mCi per mmole) from New England Nuclear, Montreal, Canada. Radiopurity, determined by thin layer chromatography and gas-liquid chromatography of the methyl esters, was more than 99% for stearic acid, more than 98% for palmitic acid, and more than 99% for stearoyl-CoA. Nonradioactive fatty acids and phospholipids were purchased from the Hormel Institute, Austin, Minn., and Applied Science Laboratories, State College, Pa.; nonradioactive stearoyl-CoA,
α-glycerophosphate, and pyridine nucleotides were obtained from Sigma Chemical Company, St. Louis, Mo. The nonradioactive stearoyl-CoA was 75% pure as determined by ratios of molar extinction coefficients at 232 nm and 260 nm (29). The radioactive substrates were diluted with the nonradioactive substrates to a specific activity of 1.8 mCi per mmole for stearic acid, 0.8 mCi per mmole for palmitic acid, and 0.7 mCi per mmole for stearoyl-CoA. Fatty acids were solubilized in a 1% aqueous solution of defatted bovine serum albumin (Calbiochem, Inc., Los Angeles, Calif.) (12) and stearoyl-CoA was added as an aqueous solution. All solvents were ACS grade and were further purified and distilled before use (24). BF3-methanol reagent (25) was prepared with BF3 from the Matheson Company of Canada, Whitby, Ontario.

Preparation of Tissues—Ten-day-old and adult (4- to 12-month-old) Woodlyn-Wistar rats of either sex were decapitated; the brains were removed immediately and were homogenized in a glass tube, using a Teflon pestle, with 0.32 M sucrose containing 1.0 mM EDTA. Subcellular fractionation was carried out in a glass tube, using a Teflon pestle, with 0.32 M sucrose containing 1.0 mM EDTA. Subcellular fractionation was carried out as previously described (26), and protein content was determined by the method of Lowry et al. (27).

Incubation and Extraction—Except where indicated otherwise, the incubation mixture with BSA-complexed fatty acids was 3.5 mM ATP, 0.1 mM CoA, 2.4 mM MgCl2, 0.7 mM NADH, 7.5 mM phosphate buffer at pH 7.4, 100 to 200 μl labeled fatty acid substrate, rat brain homogenate containing 8 to 12 mg of protein, and H2O to a total volume of 2.1 ml. For stearoyl-CoA incubation (50 to 75 μM in aqueous solution), ATP, CoA, and MgCl2 were omitted from the mixture and 0.3 ml of 1% BSA solution in water was added. Incubation was at 37°C for 10 min (stearoyl-CoA) or 15 min (free fatty acids) in a shaking water bath. For fatty acid experiments, the reaction was stopped by adding 20 ml of chloroform-methanol (2:1, v/v); for stearoyl-CoA, the reaction was stopped by adding 2.0 ml of 10% KOH in methanol, heating to 55°C for 10 min, acidifying with concentrated HCl, and then adding 18 ml of chloroform-methanol (5:2, v/v). Total lipids were extracted according to the method of Folch et al. (28) and transmethylated with 10% BF3-methanol (w/v) for 90 min at 105°C (29). The fatty acid methyl esters were extracted with petroleum ether, and monoenes were separated by AgNO3-thin layer chromatography in toluene (16). After detection with 0.1% Rhodamine-6G in water, the area corresponding to a monoenic fatty-acid methyl ester standard was extracted from the silica gel with diethyl ether; the extract was dried under a stream of air in counting vials. Scintillation fluid (10 ml toluene containing 0.4% 2,5-di-phenyloxazole (PPO) and 0.01% 1,4-bis[2-(5-phenyloxazolyl)]benzene (POPOP)) was added, and radioactivity was measured in a Nuclear Chicago Mark II liquid scintillation spectrometer; efficiency was determined with an external standard.

Identification of Fatty Acids—Fatty acid methyl esters were identified by a combination of thin layer chromatography and gas-liquid chromatography. The gas-liquid partition chromatograph (model 851; Perkin-Elmer Corp., Norwalk, Conn.) was equipped with a hydrogen flame ionization detector and contained a 6-foot stainless steel column, 3.2 mm outside diameter, packed with 15% diethylene glycol succinate polyester on Chromosorb W, 60 to 80 mesh; helium flow rate, 87 ml per min; column temperature, 180°C. Fatty acid methyl esters were identified by comparing their retention time with that of known standards and by plotting log retention time. The radioactive methyl esters in the effluent gas were collected in glass tubing containing Dowex 2-X8 resin coated with 10% Dow Corning 200 fluid, using a gas fraction collector (model 503; Packard Instrument Co., Downer’s Grove, Ill.). Tubing and contents were placed in scintillation vials, and a 10-ml aliquot of scintillation mixture was added before counting. Recovery of labeled fatty acid methyl esters was routinely greater than 80%.

Oxidation of Double Bonds—Preparative gas-liquid chromatography, sample collection, and periodate-permanganate oxidation were carried out as previously described (30). Dicarboxylic acid dimethyl esters were analyzed by gas-liquid chromatography and identified by reference to standard mixtures.

RESULTS

Optimal Incubation Conditions

pH, Temperature, Enzyme Concentration, and Time—Optimal pH for the formation of monoene from stearic or palmitic acids in brain homogenate from 10-day-old rats was 7.2 to 7.6; phosphate and citrate-phosphate buffers were used to show that desaturation of stearoyl-CoA is maximal at 6.0 to 6.6 (Fig. 1). The optimal temperature for monoene formation from stearic or palmitic acid was 37 to 45°C, and from stearoyl-CoA at pH 6.0 and pH 7.4 was 37 to 40°C (Fig. 2A); in all cases, activity was greatly reduced at 23°C (ambient temperature) and 50°C and was not detectable with incubation at 0°C. The reaction was linear with protein concentration between 5 and 10 mg per 2.1 ml for both stearic acid and stearoyl-CoA (Fig. 2B); inhibition usually occurred at protein concentrations more than 15 mg per 2.1 ml of incubation medium, and measured activity varied at less than 5 mg of protein. Incubations with stearic acid were stopped at 15 min and with stearoyl-CoA at 10 min, while the time response of the enzyme was linear (Fig. 2C); monoene production declined sharply after 90 min and was insignificant by 45 min.

Other Factors—Incubation in an O2-CO2 (95:5) atmosphere did not appreciably affect monoene formation with stearic acid as substrate (control, 23.9; O2-CO2, 20.2 pmol per min per mg of protein), but N2 decreased it to 4.7. The addition of ascorbic acid (100 μM), Fe2+ (10 μM FeSO4), Fe3+ (10 μM FeCl3) reduced the rates to 21.4, 14.5, and 16.9 pmol per min per mg of protein, respectively. Coenzyme A, ATP, and Mg2+ were nec-

Fig. 1. Effect of pH on rate of monoene formation by homogenates of 10-day-old rat brain. •—•, stearic acid; ○—○, palmitic acid; △—△, stearoyl-CoA. Both phosphate and citrate-phosphate buffers were used in the pH range 5 to 7. The incubation medium after all additions was within 0.2 pH unit of the buffer.

† The abbreviation used is: BSA, bovine serum albumin.
necessary for maximal activity with stearic acid (Fig. 3) but not with stearoyl-CoA. NADH was necessary for maximal desaturation of stearic acid and of stearoyl-CoA. For all of these components, concentrations higher than necessary for maximal activity did not inhibit the reaction and were used in all experiments. In 10-day-old brain homogenates, with stearic acid, palmitic acid, or stearoyl-CoA as substrate, and in adult brain microsomes with stearic acid as substrate, NADH and NAD+ were the most effective nucleotides and NADPH and NADP+ were less effective (Table I). The effect of NADH combined with NADPH, or of NADH with NAD+, was not additive. When nucleotide was not added, desaturation was still appreciable in brain homogenate, probably due to endogenous nucleotide, but was not detectable in microsomes prepared from adult rat brain.

**Substrate Concentration**—Stearic acid was more actively desaturated than was palmitic acid (Fig. 4); for both free fatty acids, reaction velocity became independent of substrate concentration at more than 200 μM. With stearoyl-CoA, the optimal substrate concentration was approximately 60 μM at both pH 6.0 and pH 7.4. Because of inhibition at high substrate concentrations and variation at very low concentrations, it was difficult to determine \( K_m \) for the desaturation reactions. Estimates of apparent \( K_m \) (μM) and maximal velocity (pmoles per min per mg of protein) respectively were 62 and 48 for stearic acid, 169 and 33 for palmitic acid, 28 and 46 for stearoyl-CoA at pH 7.4, and 21 and 62 for stearoyl-CoA at pH 6.0. Most assays were carried out at substrate concentrations along the shoulder of the curves, allowing recovery of radioactively labeled product significantly above background values and avoiding the possibility of substrate inhibition. Further, the use of these concentrations decreases the likelihood of non-first order reaction conditions, of reduced substrate availability by incorporation into lipids, or of significant substrate dilution by endogenous fatty acids (13).

**Other Properties**

**Enzyme Instability**—Desaturation activity declined in whole tissues stored at -20°C for more than 2 weeks and in homogenates kept at 0 to 4°C for more than 30 min before incubation, especially in tissues incubated with stearoyl-CoA at pH 6.0. A similar effect was observed in homogenates frozen to -70°C in methanol-Dry Ice and then rapidly thawed before incubation, especially when they were held for 90 min at 0°C before incubation. The addition of reagents that might react at potential sites of ac-

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**Fig. 2.** Rate of monoene formation by homogenates of 10-day-old rat brain. ○—○, stearic acid at pH 7.4; △—△, stearoyl-CoA at pH 6.0; ■—■, stearoyl-CoA at pH 7.4. A, effect of temperature. B, effect of enzyme concentration. Protein concentration (per 2.1 ml of final incubation volume) was varied by dilution of homogenates with homogenizing medium. C, effect of time.

**Fig. 3.** Effect of concentrations of ATP (A), coenzyme A (B), MgCl₂ (C), and NADH (D) on the rate of stearic acid desaturation by homogenates of 10-day-old rat brain.

**Table I**

<table>
<thead>
<tr>
<th>Nucleotide</th>
<th>Homogenate: 10-day-old brain</th>
<th>Microsomes: adult brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stearic acid</td>
<td>13.0</td>
<td>7.1</td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>18.8</td>
<td>14.0</td>
</tr>
<tr>
<td>Stearoyl-CoA</td>
<td>15.5</td>
<td>9.4</td>
</tr>
<tr>
<td>None</td>
<td>17.4</td>
<td>12.3</td>
</tr>
</tbody>
</table>

* Oxidized form, confirmed by fluorescence (31) and absorption spectra.
FIG. 4. Effect of substrate concentration on desaturation by 10-day-old rat brain homogenate. O, stearic acid at pH 7.4; △, palmitic acid at pH 7.4; ●, stearoyl-CoA at pH 6.0; □, stearoyl-CoA at pH 7.4. Homogenates containing 7 to 10 mg of protein were incubated at 37°C with a constant amount of labeled substrate and various concentrations of unlabeled substrate.

Activity or protect essential functional groups on the enzyme did not prevent this loss (Table II) and, in general, resulted in inhibition, although with most reagents the decrease was slightly less when stearoyl-CoA was used as substrate. Only niacinamide with hydroquinone appreciably protected the enzyme from loss of activity, but this mixture depressed activity in homogenates incubated immediately and therefore could not be used. This instability probably explains our failure to obtain significantly enriched enzyme activity in subcellular fractions of 10 day old rat brain. Rapid subcellular fractionation, variation in fractionation methods (including the use of sucrose gradients), and temperature control (0 to 4°C) resulted in an enriched microsomal pellet in adult rat brain only. Therefore, for all brain studies we used a crude homogenate of fresh tissue or tissue stored at -20°C for less than 2 weeks, prepared less than 1 min before incubation.

**Acceptor for Product—**Labeled fatty acid or fatty acyl-CoA (predominantly the saturated substrate) was actively incorporated into complex lipids in 10-day-old rat brain homogenates (Table III). The rate of incorporation into phosphoglycerides, used as a measure of acyltransferase activity (acyl-CoA:sn-glycero-phosphatide transferase), greatly exceeded the rate of desaturation of stearic acid or stearoyl-CoA at either pH 6.0 or 7.4. With either substrate, acyltransferase activity was decreased at pH 6.0, the optimal pH for desaturation of stearic acid or stearoyl-CoA. As labeled stearic acid or stearoyl-CoA incorporated most actively into choline phosphoglyceride, we tested lyssolecithin (1-acyl-sn-glycerol-3-phosphorylcholine) as a product acceptor in the incubation medium with 10-day-old brain homogenate and stearic acid as substrate; sn-glycerol-3-phosphate also was tested. The effect of lyssolecithin was varied; at low concentrations (10 to 40 µM, suspended in the incubation medium by vigorous shaking) it was stimulatory in some experiments and inhibitory in others, and at high concentrations (140 to 285 µM) it was slightly but consistently inhibitory (less than 5%). When it was complexed to BSA before addition to the incubation medium there were similar variations. (Phosphorus analysis revealed that more than 85% of the lyssolecithin was suspended in the albumin solution.) At the three concentrations tested (0.03, 0.1, and 5.0 mM), sn-glycerol-3-phosphate slightly inhibited activity (less than 11%).

**Product Inhibition—**Product inhibition was determined by the addition of individual monoenes to the incubation medium (Table IV). Palmitoleic acid (16:1(n-7)), at all concentrations tested, inhibited desaturation of both stearic and palmitic acids. Oleic acid (18:1(n-9)) had varied effects; desaturation of stearic acid and of stearoyl-CoA was slightly stimulated at low concent-

### Table II

<table>
<thead>
<tr>
<th>Reagents added to homogenizing medium</th>
<th>Stearic acid</th>
<th>Stearoyl-CoA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (0.32 M sucrose and 1 mM EDTA alone)</td>
<td>28.1</td>
<td>12.3</td>
</tr>
<tr>
<td>p-Hydroxymercuribenzoate, 0.1 mM</td>
<td>24.7</td>
<td>6.7</td>
</tr>
<tr>
<td>Dithiothreitol, 0.5 mM; reduced glutathione, 0.3 mM</td>
<td>22.7</td>
<td>10.0</td>
</tr>
<tr>
<td>Niacinamide, 0.6 mM; hydroquinone, 0.02 mM</td>
<td>17.4</td>
<td>24.5</td>
</tr>
<tr>
<td>Dithiothreitol, 0.5 mM; reduced glutathione, 0.3 mM; niaconamide, 0.6 mM; hydroquinone, 0.02 mM</td>
<td>25.0</td>
<td>11.0</td>
</tr>
<tr>
<td>Phosphate buffer, 15 mM, at pH 7.4*</td>
<td>20.2</td>
<td>18.8</td>
</tr>
<tr>
<td>Phosphate buffer, 15 mM, at pH 8.6*</td>
<td>14.1</td>
<td>12.1</td>
</tr>
</tbody>
</table>

* Before incubation the homogenate was frozen to -70°C (methanol and solid CO₂), then thawed to 0 to 4°C and held at that temperature for 60 min.
* Sucrose and EDTA were omitted from the homogenizing medium.

### Table III

**Comparison of desaturation and transferase activity with stearic acid and stearoyl-CoA at pH 7.4 and 8.0**

In incubations were as described under "Experimental Procedure." For transferase activity, an aliquot of the lipid extract before transmethylation was chromatographed, using two-dimensional thin layer chromatography with chloroform-methanol-water (50:20:10:10:5) and chloroform-acetone-methanol-glacial acetic acid-water (50:20:10:10:5). Complex lipids include choline, serine, ethanolamine, and inositol phosphoglycerides, sphingomyelin, lysophosphatidyl choline, and neutral lipids. All values are the average of duplicate determinations.

<table>
<thead>
<tr>
<th>pH</th>
<th>Substrate</th>
<th>Desaturation (pmoles/mg protein)</th>
<th>Transferase (incorporation into complex lipids) (pmoles/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.4</td>
<td>Stearic acid</td>
<td>25.6</td>
<td>280</td>
</tr>
<tr>
<td>7.4</td>
<td>Stearoyl-CoA</td>
<td>29.8</td>
<td>372</td>
</tr>
<tr>
<td>6.0</td>
<td>Stearic acid</td>
<td>3.0</td>
<td>105</td>
</tr>
<tr>
<td>6.0</td>
<td>Stearoyl-CoA</td>
<td>49.0</td>
<td>148</td>
</tr>
</tbody>
</table>
Effect inhibitory effects were observed at concentrations of monoenoic fatty acid higher than those in normal brain tissue. Stimulation of desaturation at low concentrations and inhibition at high ones. The effects of cis-vaccenic acid on stearic acid, palmitic acid, and undecanedioic acid (C_{11}) also varied; stearic acid desaturation was little affected, and palmitic acid desaturation was stimulated at low concentrations but inhibited at higher ones. (These effects were not further examined. The important point is that all inhibitory effects were observed at concentrations of monoenoic fatty acid higher than those in normal brain tissue in vivo (32, 33) or produced by our system in vitro.)

**TABLE IV**

Effect of monoenoic fatty acids on stearic acid, palmitic acid, and stearoyl-CoA desaturation by homogenates of 10-day-old rat brain.

All incubations were carried out at pH 7.4. Test monoenoic fatty acids were suspended in 1% BSA.

<table>
<thead>
<tr>
<th>Monoene added</th>
<th>Concentration (nM)</th>
<th>Substrate</th>
<th>Products monoene formed/ min/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>Stearic acid</td>
<td>29.4</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>5</td>
<td>23.0</td>
<td>17.6</td>
</tr>
<tr>
<td>Palmitoleic acid</td>
<td>25</td>
<td>24.9</td>
<td>12.9</td>
</tr>
<tr>
<td>cis-Vaccenic acid</td>
<td>50</td>
<td>24.3</td>
<td>16.5</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>21.4</td>
<td>21.7</td>
</tr>
</tbody>
</table>

**DISCUSSION**

In mammalian tissues the formation of monoenoic from free fatty acids is a two-stage reaction—activation of the saturated fatty acid to fatty acyl-CoA by acyl-CoA synthetase (acid:CoA ligase [AMP] EC 6.2.1.3), followed by desaturation of the fatty acyl-CoA derivative (14). Most previous studies of desaturation used free fatty acid as substrate, and thus measured the rate of both reactions. In the present study with rat brain tissue, the two-stage reaction was measured with stearic acid as substrate; also, desaturation was studied as a single reaction, using stearoyl-CoA as substrate.

Whereas the optimal pH for the two-stage reaction was 7.2 to 7.6, the pH optimum for the desaturation reaction per se was 6.0 to 6.6, a pH that apparently was not used in previously reported studies of stearoyl-CoA desaturation (34–36). Monoene formation with both stearic acid and stearoyl-CoA was measured at pH 7.4 to see whether acyl activation or desaturation was the rate-limiting step in the two-stage reaction, and also with stearoyl-CoA at pH 6.0, the optimum for stearoyl-CoA desaturation. When stearic acid was used as substrate, the components necessary for acyl activation of free fatty acid (ATP, Mg^{2+}, and coenzyme A) were added in optimal concentrations (Fig. 3) to ensure maximal acyl-CoA synthetase activity. Clear delineation of the experimental findings as due to desaturation and not to acyl activation was possible in most cases.

Comparison of the substrate curves and apparent K_m and V_max values for desaturation of stearic and palmitic acids shows that, under optimal conditions, stearic acid was more actively desaturated than palmitic acid by the coupled enzyme system of 10-day-old brain (Fig. 4). This is consistent with the increased requirement for products of stearic acid desaturation as the brain matures (15) and agrees with the reported findings that 10% of the stearic acid and less than 5% of the palmitic acid in brain tissue explants was desaturated by 48 hours (22). Monoene formation was inhibited by high concentrations of stearic or palmitic acid and even more by stearoyl-CoA (Fig. 4). At constant levels of substrate, monoene formation was inhibited at high protein concentrations and slowed by prolonged incubation (Fig. 2). These latter two findings, observed in other desaturation systems also (11), are not due solely to the addition or ac-
cumulation of desaturation product (37), as the concentration of oleic acid (18:1(n-9)) required to inhibit desaturation of stearic acid or stearoyl-CoA (Table IV) was much greater than that in the homogenate or produced by the enzyme.

The extreme sensitivity of the desaturation system makes storage of brain tissue a problem and critically affects attempts to determine subcellular location of enzymatic activity. Various treatments of brain homogenates generally affected stearic acid desaturation more than stearoyl-CoA desaturation (Table II); however, the similar direction of these effects, and the marked loss of activity with either substrate after prolonged storage, suggests that these conditions principally affect the desaturation reaction rather than acyl activation. A lipid cofactor similar to that shown to be essential for desaturation by liver enzyme (38) may be disrupted or removed during manipulation of brain tissue; such an alteration may lead to enzyme instability, and may explain our failure to obtain an enriched subcellular fraction from 10-day-old brain. The high speed particulate fraction from adult brain was somewhat enriched, suggesting that the desaturation system was in the microsomal fraction of brain tissue.

Several monoene-forming systems are known to require molecular oxygen and reduced pyridine nucleotide to desaturate the CoA derivative of the fatty acid (1, 8, 34). In the present study (Table I), in 10-day-old brain homogenate and adult brain microsomes the reduced and oxidized nucleotides were equally effective, the diphosphonucleotides being more active than triphospho-forms. The rate of monoene formation after addition of NADH with NAD+ or NADPH was similar to that after adding NADH alone with either stearic acid or stearoyl-CoA as substrate. In view of the findings cited above (1, 8, 34), the equal but nonadditive effect of NAD+ and NADH in brain homogenates and microsomes suggests that, in the absence of NADH, NAD+ is sufficiently reduced to meet the requirements for optimal desaturation. However, the roles of oxygen and pyridine nucleotides in the mechanism of fatty acyl-CoA desaturation are not known (see References 4, 34, 35, 39), and it should be borne in mind that oxidized pyridine nucleotide may be directly involved in the desaturation reaction by brain.

Few studies of stearic and palmitic acid desaturation by mammalian tissues have identified the position of the double bond in the monoenes formed. In our experiments, oleic acid (18:1(n-9)) was the major product of stearic acid or stearoyl-CoA desaturation by 10-day-old brain, a finding similar to that reported for adult rat liver (7, 40); a small amount of 20:1, insufficient for isomer characterization, was formed by chain elongation. Palmitic acid, a precursor of palmitoleic acid (16:1(n-7)) and cis-vaccenic acid (18:1(n-7)) in rat liver (40), was converted to 22% palmitoleic and 72% cis-vaccenic acid in brain. The absence of labeled 18:1(n-8) clearly establishes that, under the conditions of these experiments, desaturation (16:0 $\rightarrow$ 16:1(n-7)) preceded chain elongation (16:1(n-7) $\rightarrow$ 18:1(n-7)); further, the preponderance of 18:1(n-7) indicates that chain elongation proceeded very actively. Thus desaturation, coupled when necessary with chain elongation, can supply the major monoene isomers required by maturing brain. Further, the relative amounts of 18:1(n-9) and 18:1(n-7) formed by desaturation and elongation in 10-day-old brain resembled their proportions in total rat brain phospholipids (15). This suggests that the ratios of isomers formed by endogenous biosynthesis may determine, at least partly, isomer ratios in complex lipids.

Thus, with either stearic or palmitic acid the double bond was introduced mainly between C4 and C6. Furthermore, variation in temperature, pH, and nucleotide type caused similar changes in desaturation rate with both substrates, strongly suggesting that the same enzyme acts on both fatty acids. Brett et al. (6), who examined four other desaturation systems, concluded that a widely distributed single enzyme, whose activity is maximal with stearic acid and high with palmitic acid also, inserts the double bond between C4 and C6. Our findings indicate a similar enzyme in brain.

Several investigators (19-22) have indicated that brain contains an enzyme system that forms monoenes, and the present study showed that desaturation is a significant process in 10-day-old rat brain. However, the role of this brain desaturation system relative to such extracerebral sources of monoenes as liver and diet is still unresolved. It is clear that the central nervous system does not depend solely upon extracerebral sources. Desaturation by brain, coupled with other fatty acid biosynthetic processes (i.e. de novo synthesis and chain elongation) in the brain, can supply the necessary major isomers. The brain desaturation system may assume particular importance when other supplies of monoenes are restricted and thus may play a vital role in maintaining normal brain function.

**Relationship between Desaturation and Acyltransferase**

As pointed out previously, free fatty acids must be activated to fatty acyl-CoA before desaturation can occur. The acyl-CoA is also a substrate for acyltransferase (acyl-CoA:acyl-sn-glycero-phospholipid transferase), which esterifies the fatty acid to complex phospholipids.

Comparison of activities of the desaturating system and the acyltransferase system at pH 7.4 and 6.0 is instructive. (In Table III, the substrate for acyltransferase is predominantly saturated fatty acyl-CoA, but desaturation products also are esterified by the enzyme. In these experiments, acyltransferase was measured under suboptimal conditions; for instance, exogenous lysophospholipid was not added to the incubation medium.)

1. At pH 7.4, with added CoA, ATP, and Mg++, the desaturation rate was the same with stearic acid or stearoyl-CoA, indicating that it was not limited by acyl activation. By contrast, acyltransferase activity was approximately 10 times desaturation activity with stearic acid as substrate and was even more active with stearoyl-CoA, indicating slight limitation by acyl activation.

2. At pH 6.0, the optimum for stearoyl-CoA desaturation, acyl activation was clearly rate-limiting, since desaturation of stearic acid was only 6% that of stearoyl-CoA; acyltransferase activity was much less than at pH 7.4 and probably was still limited by acyl activation, since stearoyl-CoA was more rapidly esterified than stearic acid. Even so, the rate of acyltransferase activity with stearic acid as substrate was twice that of stearoyl-CoA desaturation, implying that the rate of stearoyl-CoA formation from stearic acid was at least twice its desaturation rate.

It seems inconsistent that, despite adequate acyl synthetase activity, stearic acid desaturation should be so low at pH 6.0, the optimal pH for stearoyl-CoA desaturation. A possible explanation is that the fatty acyl-CoA derivatives available to the transferase may not be freely available for desaturation; this may imply activation by separate enzymes. Alternatively, the rate of desaturation in brain may be regulated by competition for acyltransferase for activated fatty acids, in which case the use of optimal conditions for both enzymes should reveal a
proportional change in substrate utilization when the pH is altered from 7.4 to 6.0. Since no appreciable effect on desaturation was observed when such acyl-CoA acceptors as lysolicetin and sn-glycerol-3-phosphate were added to the incubation medium, the latter direct relationship between desaturation and acyltransferase seems less likely.

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