CONVERSION OF SQUALENE INTO STEROLS BY MICROSOMAL FRACTIONS FROM BRAINS OF DEVELOPING RATS

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Received 25 October 1971

1. Introduction

It has been suggested that cell-free preparations from brain tissue are unable to convert mevalonic acid into sterols because of a metabolic block between squalene and desmosterol [1, 2]. Such a block could result either from the presence of an inhibitor substance or from an enzyme instability [2]. This present study demonstrates that microsomal fractions from brains of immature rats are capable of converting squalene into sterols *in vitro* but that the conversion requires the 100,000 *g* supernatant fraction from liver. It is suggested that the 100,000 *g* supernatant fraction from brain lacks an activator similar to that present in the liver fraction, and that this deficiency is responsible for inability of brain fractions to convert squalene into sterols. The possibility that an inhibitor is present in the brain microsomal fraction is ruled out by the data presented.

2. Materials and methods

DL-2-^{14}C-mevalonic acid as the dibenzylethylenediamine salt (specific activity 12.5 mCi/mmole) was purchased from New England Nuclear Corp., Boston, Mass. ^{14}C-Squalene was prepared by the method of Tchen [3] and purified on alumina column.

Brains and livers were obtained from Sprague-Dawley rats which were 5–12 days old. The tissues were homogenized in 3 vol of 0.02 M phosphate buffer pH 7.4 containing 0.1 mM EDTA. Homogenates were centrifuged at 800 *g* for 10 min to prepare an 800 *g* supernatant (S_1), at 18,000 *g* for 20 min to prepare an 18,000 *g* supernatant (S_2), and at 100,000 *g* for 60 min to prepare a 100,000 *g* supernatant (S_3). The microsomal pellet (MS) which remained after centrifugation at 100,000 *g* was resuspended in a volume of the phosphate buffer equal to that of the tissue homogenate. The various fractions were incubated with ^{14}C-squalene for 2 hr in an atmosphere of 95% O_2 and 5% CO_2 at 37°. Other details of the incubations are given in the legend to table 1. Incubations were terminated by addition of 15% KOH in 50% ethanol. The contents of the flask were saponified, and nonsaponifiable lipids were extracted from the saponification mixtures by light petroleum. 3β-Hydroxysterols were precipitated as digitonides. An aliquot of the light-petroleum fractions was evaporated to dryness, and the residue was then dissolved in 3 ml of acetone–ethanol (1:1) containing unlabeled lanosterol and cholesterol. The sterols were precipitated from the solvent with 7 ml of 0.5% digitonin in 50% ethanol. Digitonides were recovered by centrifugation and washed as described elsewhere [4]. Sterols were separated by thin layer chromatography (TLC) on precoated silica gel film using benzene:ethylacetate 95:5 as developing solvent. The ^{14}C content in the nonsaponifiable lipids, digitonin precipitable sterols and the TLC spots were determined in a liquid scintillation counter.

3. Results

The radioactivity scan obtained after extraction

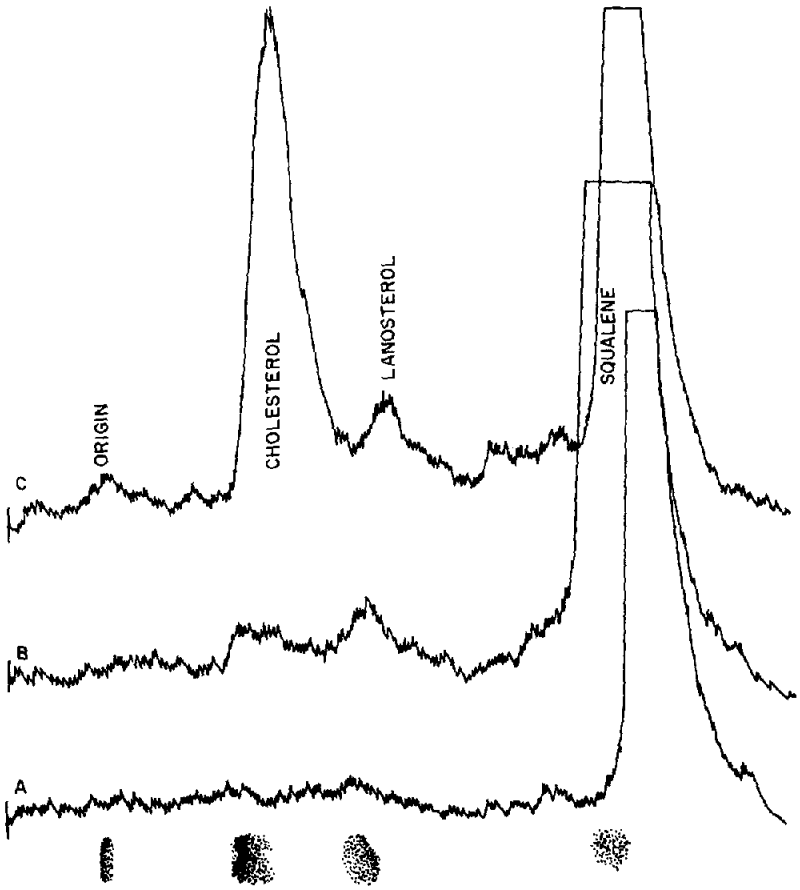

Fig. 1. Separation of squalene and sterols by thin layer chromatography. The incubation conditions were as in table 1. A) Radioactivity scan of the nonsaponifiable lipid extract obtained from a zero-time control sample for 800 *g* supernatant from liver. B) Radioactive scan of the lipid extract obtained after incubation of squalene, with 1 ml of 800 *g* supernatant fraction from brain. C) Radioactive scan of the lipid extract obtained after incubation of squalene with 1 ml of 800 *g* supernatant fraction from liver.

of nonsaponifiable lipids, and separation of squalene and sterols by TLC (fig. 1) shows that little conversion of squalene into sterols occurred when squalene was incubated with 1 ml of the 800 *g* supernatant from brain (fig. 1B). However, the incubation of squalene with 1 ml of the 800 *g* supernatant from liver showed a significant conversion into sterols, principally cholesterol (fig. 1C). The data in table 1, obtained from a representative experiment gives the quantitative distribution of ^{14}C after incubation of labeled squalene with combination of various tissue fractions. After incubation of squalene with 0.5 ml of the 800 *g* supernatant from brain, 0.68% of the label was recovered in lanosterol and 0.64% in the cholesterol fraction. When 0.5 ml of the 800 *g* supernatant from liver was substituted for the brain fraction, 1.95% and 6.32% of the label was recovered in lanosterol and cholesterol fractions, respectively. A corresponding difference between the 2 tissue fractions was also seen in the percent ^{14}C recovered as digitonin precipitable sterols. When squalene was incubated with combined 800 *g* supernatant fractions from brain (0.5 ml) and liver (0.5 ml), the percentage of ^{14}C recovered in the sterol fraction was significantly higher than what would be expected from incubation of squalene with the 2 fractions

Table 1
Conversion of squalene into sterols by cell-free preparations from brain and livers of developing rats.

Cell fraction incubated	^{14}C Recovered (% of total)		
	Digitonin precipitable sterols	Lanosterol fraction	Cholesterol fraction
S_1 B (0.5 ml)	0.87	0.68	0.64
S_1 B (1.0 ml)	1.02	1.08	0.62
S_1 L (0.5 ml)	6.76	1.95	6.32
S_1 L (1.0 ml)	10.6	2.70	9.83
S_1 B (0.5 ml) + S_1 L (0.5 ml)	10.3	2.81	10.9
S_2 B (1.0 ml)	0.65	1.07	0.55
S_2 L (1.0 ml)	10.0	2.35	7.64
MS B (1.0 ml)	0.36	0.36	0.40
MS B (1.0 ml) + S_3 B (1.0 ml)	0.61	0.86	0.53
MS B (1.0 ml) + S_3 L (1.0 ml)	7.55	6.62	1.21
S_3 B (1.0 ml)	0.34	0.31	0.48
MS L (1.0 ml)	1.16	0.51	1.04
MS L (1.0 ml) + S_3 L (1.0 ml)	15.3	1.90	14.2
MS L (1.0 ml) + S_3 B (1.0 ml)	1.32	1.00	1.20
S_3 L (1.0 ml)	0.24	0.35	0.30

^{14}C-Squalene (25,000 cpm) was incubated with various cell fractions as indicated. S_1, 800 *g* supernatant; S_2, 18,000 *g* supernatant; S_3, 100,000 *g* supernatant; MS, microsomal pellet 18,000–100,000 *g*; B, fractions obtained from brain homogenate; L, fractions obtained from liver homogenate. The final vol of 2.5 ml of the incubation mixture was made up by the addition of the phosphate-EDTA buffer (see Materials and methods for details). Each incubation contained 3 μmoles NADPH, 1.5 μmoles NAD, 60 μmoles nicotinamide, 10 μmoles glucose 6-PO_4 and 1 unit glucose 6-PO_4 dehydrogenase. All values are the average of closely agreeing duplicate determinations.

alone. This result would rule out the presence of an inhibitory substance in the cell-free preparations from brain, and would suggest that preparations from liver contain a factor(s) which enhances the conversion of squalene into sterols by brain fractions.

Incubation of labeled squalene with subcellular fractions from brain and liver yielded results which support the above suggestion. When squalene was incubated with a combination of brain microsomes and liver 100,000 *g* supernatant, there was significant conversion of squalene into sterols (6.62% in lanosterol and 1.21% in cholesterol). However, little conversion took place when squalene was incubated with brain microsomes alone (0.36% in lanosterol and 0.40% in cholesterol) or in combination with the 100,000 *g* supernatant from brain (0.86% in lanosterol and 0.53% in cholesterol). This enhanced conversion of squalene into sterols by brain microsomes in the presence of liver supernatant is also reflected in an approx. 10-fold increase in the percent ^{14}C recovered in digitonin precipitable sterols, over that which was recovered when brain supernatant replaced liver supernatant. The negligible conversion of squalene into sterols by the liver 100,000 *g* supernatant indicates that the fraction is enzymatically inactive. The data in table 1 also show that 100,000 *g* supernatant from brain tissue does not significantly enhance the conversion by liver microsomes of squalene into sterols. By contrast, squalene is converted into sterols by liver microsomes in the presence of liver 100,000 *g* supernatant.

4. Discussion

The conversion *in vitro* of squalene into cholesterol by washed microsomal fractions from liver requires the addition of 100,000 *g* supernatant fluid. The supernatant fraction has no enzyme activity but is thought to contain a protein which acts as a carrier [5] or activator [6, 7] of the precursors for cholesterol. The results of the present study demonstrate that brain microsomal fractions, either alone or in combination with 100,000 *g* supernatant from brain, are unable to convert squalene into sterols efficiently. However, when 100,000 *g* supernatant from liver is substituted for the supernatant fraction from brain, the conversion of squalene into sterols by brain microsomes is markedly enhanced. This indicates that liver supernatant can serve as an activator for squalene metabolism by brain microsomes. The fact that the 100,000 *g* supernatant from brain had no effect on the conversion of squalene into sterols by liver microsomes indicates that there is a deficiency of an activator in this fraction.

It was previously shown that brain desmosterol reductase is primarily a particulate bound enzyme, and that the activity of this enzyme is not enhanced by 100,000 *g* supernatant fraction from brain [8]. These results further support the contention that the brain fraction lacks an activator substance. The low incorporation of ^{14}C from mevalonic acid into digitonin precipitable sterols by cell-free preparations from brain [1, 2] can also be explained by such a deficiency.

It is concluded that cerebral microsomal fractions contain an active enzyme system for converting squalene into sterols, but that an apparent block in the *in vitro* conversion is caused by a deficiency of an activator or carrier protein in 100,000 *g* brain supernatant fraction.

Acknowledgements

This investigation was supported by grants from the National Institutes of Health No. HD-01823 and NS-09301. The expert technical assistance of Mr. John McKay is gratefully acknowledged.

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