Leucine and D-3-Hydroxybutyrate as Lipid Precursors in Developing Rat Spinal Cord and Liver

By ROBERT B. RAMSEY

Department of Neurology, St. Louis University School of Medicine,
St. Louis, MO 63104, U.S.A.

(Received 1 June 1976)

By using a labelled ketone body, D-3-hydroxy[3-¹⁴C]butyrate, or a ketone-body precursor, L-[4,5-³H]leucine, preferential labelling of spinal-cord cholesterol was demonstrated. In contrast, the phospholipid fraction was more heavily labelled in the liver. In both tissues phosphatidylcholine was the major labelled complex lipid. Incorporation of both substrates into total lipid, on a tissue-weight basis, was appreciably greater for spinal cord.

Ketone bodies have been shown to be a major carbon source for biosynthesis of structural components and for energy production in the developing brain (Hawkins *et al.*, 1971; Williamson & Buckley, 1973; Sokoloff, 1973; DeVivo *et al.*, 1975). They serve as physiological precursors of developing central-nervous-system lipid (Weng *et al.*, 1973; DeVivo *et al.*, 1973; Edmond, 1974; Patel *et al.*, 1975).

Developing spinal-cord cholesterol in particular is heavily labelled when radioactive 3-hydroxybutyrate is the precursor (Edmond, 1974). Leucine, which is degraded to acetoacetate and acetyl-CoA, can also serve as a suitable lipid precursor in the central nervous system (Kabara & Okita, 1961; Kabara, 1964; Smith, 1974). The specific contribution to lipid biosynthesis of that portion of the leucine molecule that ultimately is degraded to acetoacetate has not been previously tested in the central nervous system. Leucine labelled on C-4 and C-5 positions with ³H, i.e. specifically labelling the portion going to acetoacetate, has been used as a cholesterol precursor in other tissues (Miettinen & Penttila, 1968, 1971). It was found to be a better sterol precursor than acetate in muscle, intestine and kidney, but not liver (Miettinen & Penttila, 1971). The purpose of the present report is to assess L-[4,5-³H]leucine as a central-nervous-system sterol precursor *in vivo* and to determine the overall lipid labelling by leucine and D-3-hydroxy[3-¹⁴C]butyrate in developing rat spinal cord compared with liver over a relatively brief time.

Experimental

Rats were of a Wistar-derived strain; 2 days after birth, litters were decreased to seven animals each. When the animals were 11 days old they were each injected intraperitoneally with 1 μ Ci of D-3-hydroxy[3-¹⁴C]butyrate (32.3 mCi/mmol) or 5 μ Ci of L-

[4,5-³H]leucine (33.5 Ci/mmol) dissolved in 0.9% NaCl. Labelled substrates were from New England Nuclear Corp., Boston, MA, U.S.A. After the appropriate time the litter was killed and the spinal cords and livers were pooled for analysis. Each value given is the mean of duplicate determinations on tissues pooled from seven animals.

Lipids were extracted (Folch *et al.*, 1957) and fractionated on a silicic acid column (2 cm $\times$ 15 cm) (Rouser *et al.*, 1967). The neutral lipids were separated by t.l.c. with light petroleum (b.p. 30–60°C)/diethyl ether/acetic acid (82:18:1, by vol.) as the solvent system. Galactolipid and phospholipid fractions from the silicic acid column were examined further by t.l.c. with chloroform/methanol/water (70:30:4, by vol.) as the solvent system. After the plates were developed by exposure to I₂, the regions of interest were scraped directly into scintillation vials and counted for radioactivity (Jones *et al.*, 1975). Cholesterol dibromide derivatives were prepared by the method of Schwenk & Werthessen (1952).

Results and Discussion

Because the rate at which labelled 3-hydroxybutyrate and leucine are incorporated into spinal-cord lipids has as yet not been determined, uptake of these two precursors was assessed after three different times (Table 1). With both compounds, maximal lipid labelling was not obtained until 2.5 h after label administration. Still briefer time-periods are obviously needed to assess fully the linear rate of uptake of these precursors into developing spinal-cord lipid. Maximal labelling of liver lipids was obtained within 1 h with leucine, and between 1 and 5 h with labelled 3-hydroxybutyrate.

The radioactivity content of the spinal-cord neutral lipids increased throughout the time of the experiments with labelled 3-hydroxybutyrate, but was con-

Table 1. *Incorporation of D-3-hydroxy[3-¹⁴C]butyrate and L-[4,5-³H]leucine into lipids of spinal cord and liver of developing rats in vivo*

Tissues were extracted and chromatographed as described in the Experimental section. Duplicate assays of individual experiments were carried out. Reproducibility of duplicate results was within 3% for t.l.c. data and within 7% for other data.

Tissue	Precursor ...	Period after administration of labelled precursor (h)	10 ⁻² × Incorporation into total lipid extract (d.p.m./g tissue)		Distribution of label among major lipid classes (% of total labelled lipids)					
					Neutral lipid		Galactolipids		Phospholipids	
			3-Hydroxy-butyrate	Leucine	3-Hydroxy-butyrate	Leucine	3-Hydroxy-butyrate	Leucine	3-Hydroxy-butyrate	Leucine
Spinal cord	Expt. 1	1	138	68.4	49.9	63.6	8.5	3.3	41.6	33.1
			120	—	46.3	—	8.9	—	44.8	—
	Expt. 2	2.5	201	138	54.5	73.8	6.7	2.7	39.0	23.5
			239	—	51.8	—	8.2	—	40.1	—
	Expt. 1	5	200	140	62.4	72.2	3.8	1.1	33.8	26.7
			245	145	62.7	73.2	4.3	1.6	33.0	25.2
Liver	Expt. 1	1	10.0	56.9	47.3	29.0	—	—	52.7	71.0
			10.2	—	48.8	—	—	—	51.2	—
	Expt. 2	2.5	32.3	34.9	37.1	30.1	—	—	62.9	69.9
			28.2	—	43.9	—	—	—	56.1	—
	Expt. 1	5	15.0	24.8	47.4	25.7	—	—	52.6	74.3
			18.6	23.1	45.1	27.4	—	—	54.9	72.6

Table 2. *Distribution of label in neutral lipid and phospholipid fractions derived from spinal cord and liver of developing rats previously injected with D-3-hydroxy[3-¹⁴C]-butyrate or L-[4,5-³H]leucine*

Tissue	Precursor	...	Period after administration of labelled precursor (h)	Labelled sterol (% of total labelled neutral lipid)		Cholesterol dibromide (d.p.m./g of tissue)	Distribution of label among phospholipids (% of total labelled phospholipid)					
							Ethanolamine phospholipid		Phosphatidyl-choline		Phosphatidylserine + sphingomyelin	
				3-Hydroxy-butyrate	Leucine		3-Hydroxy-butyrate	Leucine	3-Hydroxy-butyrate	Leucine	3-Hydroxy-butyrate	Leucine
Spinal cord	1	...	1	98.4	100	1800	17.0	39.0	80.7	56.8	2.3	4.2
						2120						
	2.5	...	2.5	96.4	100	3270	17.3	29.8	74.2	65.5	8.5	4.7
						3920						
	5	...	5	97.5	100	4830	19.6	27.7	73.8	68.9	6.6	3.4
						4680						
Liver	1	...	1	33.6	76.1	99.3	39.1	25.9	58.3	69.0	2.6	5.1
						105						
	2.5	...	2.5	49.6	65.2	274	27.3	27.5	62.7	67.1	10.0	5.4
						256						
	5	...	5	53.5	50.9	151	28.0	20.7	63.6	72.6	8.4	6.7
						189						

Cholesterol dibromide formation and t.l.c. chromatography of lipids were carried out as described in the Experimental section. Duplicate analyses of individual experiments were made. Reproducibility of duplicate t.l.c. data was within 3% and of duplicate cholesterol determinations (by the cholesterol dibromide method) within 10%.

stant by 2.5 h when [4,5-³H]leucine was the precursor. Galactolipid and phospholipid labelling in the spinal cord was complete by 2.5 h after injection. Although the overall radioactive-lipid content fluctuated in the liver, the relative distribution of labelled material between the lipid classes was generally unchanged with both precursors.

The neutral lipids derived from spinal cord contained mainly radioactive sterol (Table 2). Small amounts of [¹⁴C]triglyceride were found in the experiments with 3-hydroxy[3-¹⁴C]butyrate. Determination of [¹⁴C]cholesterol content by the cholesterol dibromide method indicated that the gradual increase in labelled neutral lipids seen in the 3-hydroxy[3-¹⁴C]-butyrate-labelled spinal cords was reflected in the appearance of labelled cholesterol. The leucine-labelled tissues were not assessed in this manner. The liver neutral fractions contained substantial amounts of labelled triglyceride and free fatty acids, as well as sterol. Although the liver labelled with L-[4,5-³H]-leucine showed a loss of labelled sterol with time, that labelled with 3-hydroxybutyrate had a peak of [¹⁴C]sterol at 2.5 h, which then decreased with time. Examination of all the spinal-cord galactolipid fractions indicated only the presence of labelled cerebroside. The relative distribution of radioactivity among the various phospholipids exhibited little change with time. The phosphatidylcholine fractions of the 3-hydroxybutyrate-labelled spinal cords contained relatively more radioactive material than did the corresponding leucine-labelled fractions.

In comparing the two precursors, leucine and 3-hydroxybutyrate, and their incorporation into lipids of developing spinal cord and liver, the principal observation is that both were preferentially used for spinal-cord sterol formation. Since the degradation of leucine involves 3-hydroxy-3-methylglutaryl-CoA as a necessary intermediate, the question of how much labelled leucine is being transformed into cholesterol by the liver and transported to the spinal cord, instead of biosynthesis *in situ* from leucine or from liver-derived acetoacetate, may be posed. The central nervous system, however, appears to acquire little of its sterol from the systemic circulation (Davison *et al.*, 1959; Kritchevsky & Defendi, 1962; Svanberg, 1970). The effect of an altered pool size of circulating substrates on incorporation should also be considered. On the basis of the concentration of 3-hydroxybutyrate present in the blood of 6–10-day-old rats (Page *et al.*, 1971), the amount of labelled 3-hydroxybutyrate administered increased the circulating pool by approx. 10%. The concentration of the leucine

pool was essentially unaltered (Schimassek & Gevik, 1965).

When leucine serves as a sterol precursor, its incorporation into cholesterol may not be entirely sensitive to feedback inhibition by cholesterol (Miettinen & Penttilä, 1971). Control of 3-hydroxybutyrate use in sterol synthesis has not been examined. In view of the importance of ketone bodies, and possibly ketone-body precursors such as leucine, to the developing nervous system and their demonstrated role in cholesterol biosynthesis, their use requires further investigation.

References

- Davison, A. N., Dobbing, J., Morgan, R. S. & Wright, G. (1959) *Lancet* **i**, 658–660
- DeVivo, D. C., Fishman, M. A. & Agrawal, H. C. (1973) *Lipids* **8**, 649–651
- DeVivo, D. C., Leckie, M. P. & Agrawal, H. C. (1975) *J. Neurochem.* **25**, 161–170
- Edmond, J. (1974) *J. Biol. Chem.* **249**, 72–80
- Folch, J., Lees, M. & Sloane-Stanley, G. J. (1957) *J. Biol. Chem.* **226**, 497–509
- Hawkins, R. A., Williamson, D. H. & Krebs, H. A. (1971) *Biochem. J.* **122**, 13–18
- Jones, J. P., Rios, A., Nicholas, H. J. & Ramsey, R. B. (1975) *J. Neurochem.* **24**, 117–121
- Kabara, J. J. (1964) *Tex. Rep. Biol. Med.* **22**, 134–142
- Kabara, J. J. & Okita, G. T. (1961) *J. Neurochem.* **7**, 298–304
- Kritchevsky, D. & Defendi, V. (1962) *J. Neurochem.* **9**, 421–425
- Miettinen, T. A. & Penttilä, I. M. P. (1968) *Acta Med. Scand.* **184**, 159–164
- Miettinen, T. A. & Penttilä, I. M. P. (1971) *Ann. Med. Exp. Biol. Fenn.* **49**, 20–28
- Page, M. A., Krebs, H. A. & Williamson, D. H. (1971) *Biochem. J.* **121**, 49–53
- Patel, M. S., Johnson, C. A., Rajan, R. & Owen, O. E. (1975) *J. Neurochem.* **25**, 905–908
- Rouser, G., Kritchevsky, G. & Yamamoto, A. (1967) in *Lipid Chromatographic Analysis* (Marinetti, G., ed.), pp. 99–162, Marcel Dekker, New York
- Schimassek, H. & Gevik, W. (1965) *Biochem. Z.* **343**, 407–415
- Schwenk, E. & Werthessen, N. T. (1952) *Arch. Biochem. Biophys.* **40**, 334–341
- Smith, M. E. (1974) *J. Neurochem.* **23**, 435–438
- Sokoloff, L. S. (1973) *Annu. Rev. Med.* **24**, 271–280
- Svanberg, O. (1970) *Acta Physiol. Scand.* **80**, 45–49
- Weng, J. T., Nakamura, Y. & Spitzer, J. J. (1973) *Am. J. Physiol.* **225**, 967–971
- Williamson, D. H. & Buckley, B. M. (1973) in *Inborn Errors of Metabolism* (Hommes, F. A. & Van Den Berg, C. J., eds.), pp. 81–93, Academic Press, New York