Long-chain polyunsaturated fatty acids in the mammalian brain

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Introduction

The lipid-rich nature of the brain has been recognized for a long time and it is thought that the presence of myelin in the brain accounts for most of the lipid. However, brain grey matter is also rich in lipid (mostly glycerophospholipids) by comparison with other tissues (Svennerholm, 1968).

The polyunsaturated fatty acids (PUFA) associated with tissue glycerophospholipids are derived from both the linoleic and linolenic acid series. There is a tissue and species variation in the types of PUFA and in the ratio of total ω6:total ω3 fatty acids (FA) in glycerophospholipids. This generalization appears to be valid for all tissues except the grey matter of the brain where, in a wide variety of different mammals, only three major PUFA occur: these are 20:4ω6, 22:4ω6 and 22:6ω3 (Table 1). Even in marine mammals, where the ratio, dietary ω6:ω3 FA may be as low as 0·1, there is a similar profile in the brain PUFA (Bernhard, Lesch & Neuhaus-Meier, 1969; Lesch, 1969). Another feature of the brain lipids is the low level of linoleic acid compared with the levels in tissues such as liver and muscle.

Table 1. *Polyunsaturated fatty acids (mg/g total fatty acids and aldehydes) in the ethanolamine phosphoglycerides of mammalian liver and brain grey matter*

(Mean values and ranges for twenty-five species)

Fatty acid	Brain	Liver
18:2ω6	12 (3– 24)	120 (31–470)
20:3ω6	7 (2– 10)	11 (3– 45)
20:4ω6	120 (69–150)	130 (41–210)
22:4ω6	63 (42– 80)	10 (1– 56)
22:5ω6	12 (2– 29)	5 (1– 14)
18:3ω3	5 (1– 10)	21 (1– 54)
20:5ω3	6 (1– 12)	23 (5– 78)
22:5ω3	7 (3– 19)	54 (3–110)
22:6ω3	220 (160–290)	98 (2–220)

Incorporation of FA into the brain

In view of the specificity of the grey matter glycerophospholipids for long-chain PUFA it is relevant to investigate the incorporation of different FA into the brain. Several workers have shown that dietary linoleic acid is incorporated into weanling and adult rat brain lipids (Dhopeshwarkar, Subramanian & Mead, 1971; Sinclair,

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1975). Therefore the low concentration of brain linoleate is apparently not a reflection of a lack of uptake of this acid. Radioactivity from dietary linolenic acid is incorporated into the brain but most of the activity in the PUFA is associated with the 22:5 and 22:6 fraction (Table 2; Sinclair, 1975).

Dietary FA 20:4 and 22:6 are also incorporated into the developing rat brain, but in these experiments the uptake of radioactivity is considerably greater than the uptake of label when either linoleic or linolenic acids is administered (Table 2; Sinclair, 1975). It has been found that the extent of uptake of radioactivity from dietary 18:3ω6 and 20:3ω6 is proportional to the extent to which these FA are metabolized to and found in the brain as arachidonic acid (Table 2).

Table 2. *Incorporation of radioactivity from orally administered fatty acids (FA) into brain lipids of 16-d-old rats**

Labelled FA	No. of rats	Percentage of dose in brain phospholipid FA as: Total FA	18:2	γ-18:3	20:3	20:4
[1-^{14}C]18:2	6	0·37	0·11	0·01	0·02	0·09
[1-^{14}C]γ-18:3	4	0·34		0·02	0·03	0·14
[1-^{14}C]20:3	4	0·76			0·13	0·32
[1-^{14}C]20:4	3	2·51				1·76
		Total FA	α-18:3	20:5	22:5	22:6
[1-^{14}C]α-18:3	4	0·21	0·01	0·01	0·01	0·03
[U-^{14}C]22:6	4	2·30				1·79

*Rats were killed 22 h after dosing.

These results show that a number of different FA are incorporated into the developing rat brain but there appears to be a preferential incorporation of the long-chain PUFA. This is consistent with the observation that the desaturation activity (18:2ω6 to 18:3ω6) in rat brain is very low soon after birth (Strouvé-Vallet & Pascaud, 1971). This means that the longer-chain PUFA must be derived from extra-neural sources such as the liver or diet. This result is confirmed by the observations of Mohrhauer & Holman (1963). In these experiments, linoleate or arachidonate was refed to groups of essential fatty acid (EFA)-deficient rats and it was shown that the level of 20:4 in the liver and brain lipids of the latter group significantly exceeded the level of tissue 20:4 when linoleate was refed.

The level of FA 22:4ω6 in the brain is higher than in most other tissues and this suggests that this acid is synthesized in the brain. This is confirmed by the observation that there is a very active formation of 22:4ω6 in mitochondria and synaptosomes of rat brain (Aeberhard & Menkes, 1968; Koeppen, Barron & Mitzen, 1973). Linoleic acid is incorporated into the brain but the fact that it does not accumulate (Table 1) indicates that it probably has a greater turnover than other brain PUFA. It has been suggested that most of the brain linoleate is found in the cardiolipin fraction (Sun & Horrocks, 1970).

Transport of FA to the brain

Dhopeshwarkar, Subramanian, McConnell & Mead (1972) have suggested that in the rat free FA (FFA) are the preferred form of transport of FA to the brain. If there

is a specific uptake of longer-chain PUFA by the brain then it is unlikely that these molecules are transported to the brain as albumin-bound FFA, since there is very little, if any, 20:4 or 22:6 in the FFA fraction (see Table 3). Table 3 shows that the major carriers of the longer-chain PUFA in the plasma are phosphatidylcholine, triglyceride and cholesteryl ester. In studying the incorporation of radioactivity into the brain from intravenously administered lipids, difficulties can arise with the interpretation of the results. For example, intravenously administered FFA are rapidly esterified in the liver (Dhopeshwarkar *et al.* 1972) and thus one is no longer studying the uptake of radioactivity from FFA but rather from triglycerides and glycerophospholipids. This problem can be overcome by removing the liver from the circulation for a short time (Dhopeshwarkar *et al.* 1972; Table 4). Table 4 shows that radioactivity from intravenous phosphatidylcholine or FFA injections is incorporated into the brain whereas there is very little incorporation of radioactivity from the triglycerides. In the first experiment the lack of labelling of compounds other than phosphatidylcholine suggests that this was incorporated intact in the brain.

Table 3. *Concentration of polyunsaturated fatty acids (PUFA) in rat blood plasma*

(Plasma from eight 16-d-old rats was pooled prior to analysis)

	PUFA in plasma lipids (μg/ml plasma)			
Fatty acid	CE	TAG	FFA	PC
18:2ω6	128	226	10	248
18:3ω3	10	38	7	2
20:4ω6	176	19	1	119
22:6ω3	4	13	0	31

CE, cholesteryl esters; TAG, triacylglycerols; FFA, free fatty acid; PC, phosphatidylcholine.

Table 4. *Incorporation into rat brain of radioactivity from intravenously injected labelled lipids*

(Animals were killed 30 min after the intravenous injection of the labelled compounds; female rats weighing about 160 g were used.)

Expt	Labelled lipid	No. of rats	Percentage of dose incorporated into brain lipids: Mean	Range	Major brain lipids labelled
1. SO	1-acyl-2-linoleoyl-[1-^{14}C]phosphatidylcholine*	5	0·09	0·07–0·12	PC
FH		5	0·35	0·24–0·49	PC
2. SO	[1-^{14}C]linoleic acid†	5	0·13	0·10–0·17	PC, TAG>>PE>FFA
FH		6	0·42	0·36–0·53	PC>>PE, TAG>FFA
3. FH	[1-^{14}C]trilinolein‡	3	0·05	0·04–0·07	TAG>PC

SO, sham-operated; FH, functionally hepatectomized; PC, phosphatidylcholine; TAG, triacylglycerol; PE, phosphatidylethanolamine; FFA, free fatty acids.

*Prepared by the method of Webster & Cooper (1968).

†From The Radiochemical Centre, Amersham, Bucks., UK.

‡From Applied Science Labs, State College, Pennsylvania, USA.

Experimentally induced changes in brain PUFA

Although there is little variation between species with respect to the types of PUFA in the brain, considerable variations can be effected by dietary manipulation in experimental animals. In the rat most of the PUFA in the brain accumulate during the suckling period (Sinclair & Crawford, 1972) and the retention of these in the brain is high relative to other tissues during times of stress (Joel, Ellis, Lace, Joel, Swanson & Stroemer, 1974). Therefore it is more difficult to effect a change in brain PUFA by dietary manipulation after weaning than prior to weaning. Changes can be induced by giving diets containing extremely high or low ratios of the ω6:ω3 FA (e.g. ω6:ω3≃0·1 for fish oils or ≃80 for safflower-seed oil) or by giving diets containing very low levels of PUFA (Galli, White & Paoletti, 1970; Galli, Trzeciak & Paoletti, 1971). In rats given safflower-seed oil diets some of the 22:6ω3 acid in the brain is replaced by 22:5ω6, whereas in rats given EFA-deficient diets, 20:4ω6, 22:4ω6 and 22:6ω3 in the brain are partly replaced by 20:3 and 22:3ω9. Although these diets produce modifications to the normal pattern of brain PUFA, the changes are not as pronounced as those which occur in the PUFA content of tissues such as liver and muscle (Alling, Bruce, Karlsson, Sapia & Svennerholm, 1972). In the rat some protection of the brain against diets containing little fat or with high dietary ratios of ω6:ω3 FA is afforded by the poor breeding performance of rats maintained on these diets (Tinoco, Williams, Hincenbergs & Lyman, 1971; Sinclair & Crawford, 1973).

The specificity of brain PUFA

This paper has pointed out the strict specificity of certain PUFA in the mammalian brain compared with other mammalian tissues. At present, the meaning of this specificity is not apparent. Clearly the fatty-acyl residues of membrane lipids are important in membrane structure and function (for review see McElhaney, 1974). This generalization appears to be valid with respect to the brain since diet-induced changes in the fatty-acyl residues of brain glycerophospholipids are associated with changes in the properties and functions of brain membranes (Benolken, Anderson & Wheeler, 1973; Sun & Sun, 1974). Furthermore, it has been shown that induction of EFA deficiency prior to the major brain growth spurt in rats is associated with a change in the learning ability of the rats (Caldwell & Churchill, 1966; Paoletti & Galli, 1972).

Of the PUFA in the brain, it is the 22:6ω3 which appears to be specifically concentrated there relative to other tissues (Table 1). However, it is not known whether there is a specific requirement by the brain for this acid. With dietary manipulation in rats the level of 22:6ω3 (all-*cis* 4,7,10,13,16,19-docosahexaenoic acid) in the brain can be reduced considerably and be replaced by 22:5ω6 (all-*cis* 4,7,10,13,16-docosapentaenoic acid) (Eddy, 1973). The only difference between these two FA is the one extra double bond at the methyl end of the former compound. In this respect the only difference between 20:4ω6 and 20:3ω9 (the EFA-deficiency acid) is also the presence of one extra double bond in the former acid. It has been well demonstrated that the replacement of 20:4ω6 by 20:3ω9 in

tissue lipids is associated with alterations in membrane structure and function (Holman, 1970; Chen, Lund & Richardson, 1971).

Human nutrition

In view of the important role of milk in the immediate postnatal nutrition of humans it is of interest to note that the PUFA content of six common brands of artificial milk (mg/g total FA) ranges from 27 to 352, compared with about 110 in human milk. Furthermore the ratio, ω6:ω3 FA in these milks varies from 1·5:1 up to 129:1, compared with a ratio of 2·8:1 in human milk. Therefore consideration of the foregoing observations may be relevant to human nutrition.

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