33 Lipid composition of brain microvessels

JEAN-MARIE BOURRE

- Introduction
- Changes in fatty acids in relation to the diet, and alterations during development and aging
- Lipid synthesis and energy substrates
- Lipoprotein receptors and fatty acid transport
- Various lipid-related enzymatic activities
- Protection against peroxidation
- Fatty acid synthesizing systems
- Lipid metabolism in cultured cells

Introduction

Membrane lipids containing fatty acids are important as they play a direct role in the structure and function of cells, including brain endothelial cells. Moreover, these latter cells transfer fatty acids from the blood to the brain cells. Brain is the second organ in terms of lipid content, after adipose tissue; in brain, lipids do not provide any energy, but play a structural role: they participate in the structure of membranes, and thus mediate their functions. This transfer is obligatory for essential fatty acids of dietary origin, but it is not yet known whether the essential linoleic (18:2(*n*-6)) and alpha-linolenic (18:3(*n*-3)) acids are transferred, or whether it is the longer chains that are in fact transferred (mainly arachidonic acid, 20:4(*n*-6); and docosahexaenoic acid, 22:6(*n*-3), DHA).

Thus brain microvessel metabolism controls brain membrane polyunsaturated fatty acids. This is of special importance as, for instance, alterations in dietary (*n*-3) polyunsaturated fatty acids change the fatty acid profile of brain cell membranes (neurons, astrocytes and oligodendrocytes) and subcellular fractions (myelin and nerves endings) (Bourre *et al.*, 1984), and thus alter brain structure and functions including learning performance. This has been documented in animal models (Bourre *et al.*, 1989), and in human infants. In animals, during development, different structures within the brain may vary in their capacity to synthesize DHA, and this may be correlated with the regional growth rate (Pawlosky *et al.*, 1996); in human infants fed formula milks the amount of DHA produced may not reach the DHA level observed in breast-fed newborn infants (Salem *et al.*, 1996).

Consequently, it is very important to determine how polyunsaturated fatty acids are transferred from the blood to the brain, and whether dietary fatty acids are lengthened and desaturated at the level of the blood–brain barrier, since the two essential dietary precursors (linoleic and alpha-linolenic acids) are almost absent in brain membranes, and only their derived very long polyunsaturated chains are found. It is possible that microvessels play a role in the metabolism of essential fatty acids during their transfer from the circulation to the brain (metabolism and eicosanoid formation).

Changes in fatty acids in relation to the diet, and alterations during development and aging

A diet deficient in some polyunsaturated fatty acids induces alterations in fatty acids in brain capillaries (Matheson *et al.*, 1981; Homayoun *et al.*, 1988) and choroid plexus (Homayoun *et al.*, 1988), and recovery is extremely slow after substitution of a non-deficient diet (Homayoun *et al.*, 1988). During aging (Tayarani *et al.*, 1989), the percentage of total saturated fatty acids does not change. In contrast, monounsaturated fatty acids increase and polyunsaturated fatty acids decrease.

Changes in dietary fatty acids, such as alpha-linolenic acid deficiency, alter the fatty acid composition of the brain microvessels, and the efficiency of the blood–brain barrier (Ziylan *et al.*, 1992); a diet deficient in alpha-linolenic acid induces a greater transport of sucrose from blood to brain in some brain regions, but not of alpha-aminoisobutyric acid or L-phenylalanine.

Lipid synthesis and energy substrates

A number of metabolic activities and mechanisms related to lipids have been found in brain microvessels. These vessels use ketone bodies for energy production, and lipid synthesis is altered during development (Homayoun *et al.*, 1988). More precisely, the rate of CO_2 production (an index of oxidative metabolism) from glucose is slightly higher than from aceto-acetate, D-hydroxybutyrate, acetate and butyrate. Thus, ketone bodies can be used as a source of energy by brain capillaries. All the above substrates are also used for the synthesis of lipids, which *in vitro* is suppressed by the addition of albumin in the incubation medium. The incorporation of glucose into total lipids is ten times higher than that of other precursors. However, glucose is almost exclusively found in the glycerol backbone of phospholipids, especially in phosphatidylcholine.

Ketone bodies, as well as glucose, are incorporated mainly into phospholipids, whereas acetate and butyrate are mainly incorporated into neutral lipids. The rank order contribution of the various substrates to fatty acid synthesis is: butyrate > acetate > ketone bodies > glucose. All precursors, except glucose, are used for sterol synthesis.

Lipoprotein receptors and fatty acid transport

Lipoprotein receptors are present in these microvessels (Pitas *et al.*, 1987; Martin-Nizard *et al.*, 1989; Méresse *et al.*, 1989), but not albumin receptors (Pardridge *et al.*, 1985). It has been hypothesized that the entry of polyunsaturated fatty acids into the brain correlates with the high-density lipoprotein-induced methylation of phosphatidylethanolamine and phospholipase A2 (Magret *et al.*, 1996); it has been demonstrated that the conversion of phosphatidylethanolamine into phosphatidylcholine by a sequence of three methylation reactions is stimulated by the apolipoprotein E-free subclass of high density lipoprotein (HDL_3) in isolated bovine brain capillary membranes. Both methyl transferase and phospholipase A2 activity depend on HDL_3 concentration in the medium, and are strictly dependent on the binding (HDL_3 modified by tetranitromethane is no longer able to bind to specific receptors, and does not induce activation of these enzymatic activities).

This suggests that the phosphatidylcholine pool arising from phosphatidylethanolamine could be used as a pathway for the supply of polyunsaturated fatty acids to the brain.

Various lipid-related enzymatic activities

Lipoprotein-lipase (Brecher and Kuan, 1979; Shirai *et al.*, 1986) and diacylglycerol lipase and kinase (Hee-Cheong *et al.*, 1985) have been detected in rabbit and rat microvessels. HDL-lipoprotein-sphingomyelin is taken up and utilized (Homayoun *et al.*, 1989). Arachidonyl-CoA synthetase (Morand *et al.*, 1987) and non-specific acyl-CoA synthetase (Carré *et al.*, 1989) are present and play a role in fatty acid activation. The exact importance of phosphoinositide metabolism remains to be elucidated (Joo, 1992). Brain microvessels also produce HETE (Moore *et al.*, 1990).

Protection against peroxidation

Protection systems against peroxidation are altered in brain capillaries during development and aging. Between days 7 and 60 after birth, glutathione peroxidase is constant in microvessels, while glutathione reductase increases, a similar time course is observed in brain homogenate. In contrast, catalase in microvessels decreases, as it does in brain homogenate, but the activity is higher in microvessels than in brain (Buard *et al.*, 1992).

During adulthood and aging (Tayarani *et al.*, 1987, 1989), glutathione peroxidase and glutathione reductase levels do not change in brain capillaries, but increase in cerebrum and cerebellum. Catalase declines in brain capillaries, but is stable in cerebrum and cerebellum. The activities of the three enzymes are significantly higher in brain capillaries than in cerebrum and cerebellum. Superoxide dismutase increases in brain and in isolated capillaries.

Concentrations of trace elements related to protection against peroxidations are altered during aging. Copper content in capillaries increases during development and then levels off, whereas it continues to increase in cerebrum and cerebellum. Zinc increases in brain capillaries, but not in cerebrum and cerebellum. Manganese content remains stable in brain and capillaries.

Fatty acid synthesizing systems

It has not yet been determined how cerebral microvessels participate in the uptake of essential fatty acids by the brain. Precursors (linoleic and alpha-linolenic acid) are almost absent in the brain, thus they have to be desaturated either in the liver or at the level of the blood–brain barrier or the blood–CSF barrier, since enzymatic activities in the brain are nearly nil after early development (Bourre *et al.*, 1990, 1992). In fact, the fatty acids present in cerebral membranes are not the precursors, but longer chain fatty acids, mainly arachidonic acid (20:4 (*n*-6)) and docosahexaenoic acid (22:6 (*n*-3)). The key enzyme that controls all transformations (chain lengthening and desaturation) is delta-6-desaturase, which transforms linoleic acid (18:2(*n*-6)) into gamma-linolenic acid (18:3 (*n*-6)) and alpha-linolenic acid (18:3 (*n*-3)) into stearidonic acid (18:4 (*n*-3)). Another protein, cytochrome b5 reductase, is involved in the desaturation of fatty acids.

Delta-6-desaturase was measured in rat brain microvessels and choroid plexus by incubation in the presence of radioactive linoleic acid (Bourre *et al.*, 1997). In 21-day-old animals, delta-6 desaturase was not detected in brain microvessels. In contrast, it was present in choroid plexus (about 21 pmol/min/mg protein). In comparison, the activity in brain was much lower (about 1 pmol/min/mg protein) and higher in liver (about 55 pmol/min/mg protein). Interestingly, during development the activity in choroid plexus peaked at day 6 after birth and declined slightly thereafter. These results show that delta-6 desaturase was not detectable in brain microvessels but was present in choroid plexus.

Thus, brain microvessels do not contain measurable amounts of delta-6 desaturase (by direct measurement of the enzymatic activity), in contrast with endothelial cells in culture, as determined by synthesis of long polyunsaturated chains from precursors added to the culture medium (Moore *et al.*, 1991). It can be speculated that differentiation of brain endothelial cells leads to disappearance of delta-6 desaturase. A similar hypothesis is also probably valid in skin, where delta-6 desaturase is also absent (Ziboh and Chapkin, 1988). It is known that endothelial cells in culture rapidly lose markers found in the microvessels, such as gamma-glutamyl-transpeptidase or alkaline phosphatase (Roux *et al.*, 1994).

As microvessels contain measurable amounts of very long chain polyunsaturated fatty acids, such as arachidonic and docosahexaenoic acids, these fatty acids must be supplied by the blood. It has been recently demonstrated that docosahexaenoic acid is taken up by the microvessels from developing rat brain (Anderson *et al.*, 1993). This acid synthesizes endothelial cell membrane lipids, but could be transferred to the other brain cells.

As delta-6 desaturase was not detected in brain microvessels during development, very long unsaturated chains found in nervous tissue are probably not synthesized in the microvessels; this is in agreement with the hypothesis that they are either synthesized by the liver or supplied directly by the diet (Bourre *et al.*, 1989; Scott and Bazan, 1989).

Lipid metabolism in cultured cells

Lipid synthesis in primary cultures of microvascular endothelial cells from rat brain is very active. These cells are able to synthesize all their lipids (phospholipids and neutral lipids) from various water-soluble compounds such as glucose, acetate, aceto-acetate and beta-hydroxybutyrate; the ketone bodies being the preferred substrates for lipid synthesis. The metabolic pathway is different for glucose, which is preferentially incorporated into phospholipids. The existence of an inverse relationship between serum lipoprotein levels and lipid synthesis suggests that cultured endothelial cells are able to take up lipids (such as cholesterol) contained in lipoproteins. Consequently, lipids in endothelial cells could be supplied either by intracellular synthesis or by serum lipoproteins. Activity in cultured cells is at variance with that observed in isolated capillaries: it is regulated by serum lipoproteins (Roux *et al.*, 1989).

Cultured murine cerebro-microvascular endothelia are able to convert linoleic acid into arachidonic acid, and alpha-linolenic acid into EPA but not DHA; although the uptake of linoleic and alpha-linolenic acid is similar, alpha-linolenic acid is more extensively elongated and desaturated (Moore *et al.*, 1990). The blood–brain barrier can play an important role in the elongation and desaturation of essential fatty acids during transfer from the circulation into the brain. Primary rat cultures of neurons do not produce DHA, but primary cultures of astrocytes do (Moore *et al.*, 1991). However, it is not totally certain whether results concerning fatty acid metabolism, obtained with cultured cells, can provide information about the actual situation *in vivo*. In fact, age is an important parameter, as delta-6-desaturase levels are high in brain during development, but low thereafter (Bourre *et al.*, 1990, 1992). Moreover, delta-6 desaturase is extremely low in adult brain microvessels (Bourre *et al.*, 1997).

Co-culture with astrocytes changes the fatty acid composition of bovine brain capillary endothelial cells (Bénistant *et al.*, 1995). Co-culture increases arachidonic concentration, at the expense of linoleic acid, and DHA is increased at the expense of its precursors. Interestingly, the changes induced by co-culture are found only in phospholipids (especially phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine), but not in phosphoinositides and other lipid classes. Only (n-3) fatty acids were altered in capillary endothelial cells from adrenal cortex co-cultured under the same conditions.

Table 33.1. *Linoleic acid incorporation in isolated 21-day-old brain capillaries.*

	Capillaries
Cholesterol	3.0±0.8
Cholesterol ester	4.2±1.4
Free fatty acids	19.9±4.8
Phosphatidylethanolamine	6.9±0.4
Phosphatidylinositol + phosphatidic acid	1.5±0.1
Phosphatidylserine	6.7±1.0
Phosphatidylcholine	55.8±2.7
Sphingomyelin	1.9±0.5

DHA is the major (n-3) polyunsaturated fatty acid in rat brain microvessels (Homayoun *et al.*, 1988) and in bovine retinal microvessels; its concentration is restored in bovine retinal endothelial cells in co-culture when the medium is enriched in this fatty acid (Lecomte *et al.*, 1996). The very high concentration of polyunsaturated fatty acids in retinal microvessels raises the question of the pathogenic processes leading to diabetic neuropathy through oxidation products derived from these fatty acids.

Table 33.1 shows distribution of radioactivity in the various main lipids in brain microvessels after incubation with radioactive linoleic acid. Radioactivity in sphingomyelin was low; as expected since sphingomyelin is known not to contain polyunsaturated fatty acids. The low radioactivity in cholesterol is probably due to degradation of the labelled linoleic acid to labelled acetate units, which are in turn utilized to synthesize cholesterol or saturated and monounsaturated fatty acids found in sphingomyelin. The high labelling of free fatty acids in capillaries could be because endothelial cells are low in fatty-acid-binding proteins, at least in heart, and this may result in accumulation of free fatty acids.

Interestingly, the role of choroid plexus remains to be determined. It could be proposed as an

alternative pathway for supplying brain tissue with very long unsaturated chains via the CSF lipoproteins. Thus, choroid plexus metabolism must be taken into account in the delivery of lipids to the brain, at least at the level of polyunsaturated fatty acids. It is not known whether the choroid plexus uses all the very long chain fatty acids it synthesizes or whether some are also transported to the brain. As monolayer cultures are now possible, it would be interesting to measure their delta-6 desaturase activity.

Acknowledgments

The author is most grateful to INSERM for supporting this work, and to A. Strickland for reviewing this manuscript.

References

Anderson, G. J., Hohimer, A. R. and Willeke, G. B. (1993). Uptake of docosahexaenoic acid by microvessels from developing rat brain. *Life Sci.*, **53**, 1089–98.

Bénistant, C., Dehouck, M. P., Fruchart, J. C. *et al.* (1995). Fatty acid composition of brain capillary endothelial cells: effect of the coculture with astrocytes. *J. Lipid Res.*, **36**, 2311–19.

Bourre, J. M. and Piciotti, M. (1992). Delta-6 desaturation of alpha-linolenic acid in brain and liver during development and aging in the mouse. *Neurosci. Lett.*, **141**, 65–8.

Bourre, J. M., Pascal, G., Durand, G. *et al.* (1984). Alterations in the fatty acid composition of rat brain cells (neurons, astrocytes and oligodendrocytes) and of subcellular fractions (myelin and synaptosomes) induced by a diet devoid of n-3 fatty acids. *J. Neurochem.*, **43**, 342–8.

Bourre, J. M., François, M., Youyou, A. *et al.* (1989). The effects of dietary α-linolenic acid on the composition of nerve membranes, enzymatic activity, amplitude of electrophysiological parameters, resistance to poisons and performances of learning tasks in rats. *J. Nutr.*, **119**, 1880–92.

Bourre, J. M., Piciotti, M. and Dumont, O. (1990). Delta-6 desaturase in brain and liver during development and aging. *Lipids*, **25**, 354–6.

Bourre, J. M., Dinh, L. Bolthias, C. *et al.* (1997). Possible rôle of the choroid plexus in the supply of brain tissue with polyunsaturated fatty acids. *Neurosci. Lett.*, **224**, 1–4.

Brecher, P. and Kuan, H. T. (1979). Lipoprotein lipase and acid lipase activity in rabbit brain microvessels. *J. Lipid Res.*, **20**, 464–71.

Buard, A., Clement, M. and Bourre, J. M. (1992). Developmental changes in enzymatic systems involved in protection against peroxidation in isolated rat brain microvessels. *Neurosci. Lett.*, **141**, 72–4.

Carré, J. B., Morand, O., Homayoun, P. *et al.* (1989). Purified rat brain microvessels exhibit both acid and neutral sphingomyelinase activities. *J. Neurochem.*, **52**, 1294–9.

Hee-Cheong, M., Fletcher, T., Kryski, S. K. and Severson, D. L. (1985). Diacylglycerol lipase and kinase activities in rat brain microvessels. *Biochim. Biophys. Acta*, **833**, 59–68.

Homayoun, P. and Bourre, J. M. (1987). Ketone body utilization for energy production and lipid synthesis in isolated rat brain capillaries. *Biochem. Biophys. Acta*, **922**, 345–50.

Homayoun, P., Durand, G., Pascal, G. and Bourre, J. M. (1988). Alteration in fatty acid composition of adult rat brain capillaries and choroid plexus induced by a diet deficient in n-3 fatty acids: slow recovery after substitution with a nondeficient diet. *J. Neurochem.*, **51**, 45–8.

Homayoun, P., Bentejac, M., Lecerf, J. and Bourre, J. M. (1989). Uptake and utilization of double-labeled high-density lipoprotein sphingomyelin in isolated brain capillaries of adult rats. *J. Neurochem.*, **53**, 1031–5.

Joo, F. (1992). The cerebral microvessels in culture, an update. *J. Neurochem.*, **58**, 1–17.

Lecomte, M., Paget, C., Ruggiero, D. *et al.* (1996). Docosahexaenoic acid is a major n-3 polyunsaturated fatty acid in bovine retinal microvessels. *J. Neurochem.*, **66**, 2160–7.

Magret, V., Elkhalil, L., Nazih-Sanderson, F. *et al.* (1996). Entry of polyunsaturated fatty acids into the brain: evidence that high-density lipoprotein-induced methylation of phosphatidylethanolamine and phospholipase A_2 are involved. *Biochem. J.*, **316**, 805–11.

Martin-Nizard, F., Meresse, S., Cecchelli, R. *et al.* (1989). Interactions of high-density lipoprotein 3 with brain capillary endothelial cells. *Biochim. Biophys. Acta*, **1005**, 201–8.

Matheson, D. F., Oei, R. and Roots, B. I. (1981). Effect of dietary lipid on the acyl group composition of glycerophospholipids of brain endothelial cells in the developing rat. *J. Neurochem.*, **36**, 2073–9.

Méresse, S., Delbart, C., Fruchart, J. C. and Cecchelli, R. (1989). Low-density lipoprotein receptor on endothelium of brain capillaries. *J. Neurochem.*, **53**, 340–5.

Moore, S. A., Yoder, E. and Spector, A. A. (1990). Role of the blood–brain barrier in the formation of long-chain n-3 and n-6 fatty acids from essential fatty acid precursors. *J. Neurochem.*, **55**, 391–402.

Moore, S. A., Yoder, E., Murphy, S. *et al.* (1991). Astrocytes, not neurons, produce docosahexaenoic acid (22:6 n-3) and arachidonic acid (20:4 n-6). *J. Neurochem.*, **56**, 518–24.

Morand, O., Carré, J. B., Hornayoun, P. *et al.* (1987). Arachidonyl-coenzyme-A synthetase and nonspecific acyl-coenzyme-A synthetase activities in purified rat brain microvessels. *J. Neurochem.*, **48**, 1150–6.

Pardridge, W. M., Eisenberg, J. and Cefalu, W. T. (1985). Absence of albumin receptor on brain capillaries in vivo or in vitro. *Am. J. Physiol.*, **249**, E264–E267.

Pawlosky, R. J., Ward, G. and Salem, N. (1996). Essential fatty acid uptake and metabolism in the developing rodent brain. *Lipids*, **31**, 103–7.

Pitas, R. E., Boyles, J. K., Lee, S. H. *et al.* (1987). Lipoproteins and their receptors in the central nervous system, characterization of the lipoproteins in cerebrospinal fluid and identification of apolipoprotein B,E(LDL) receptors in the brain. *J. Biol. Chemistry*, **262**,14352–60.

Roux, F. S., Mokni, R., Hughes, C. C. *et al.* (1989). Lipid synthesis by rat brain microvessel endothelial cells in tissue culture, *J. Neuropathol. Exp. Neurol.*, **48**, 437–47.

Roux, F., Durieu-Trautman, O., Chaverot, N. *et al.* (1994). Regulation of gamma-glutamyl transpeptidase and alkaline phosphatase activities in immortalized rat brain microvessel endothelial cells. *J. Cell. Physiol.*, **159**, 101–13.

Salem, N., Wegher, B., Mena, P. and Uauy, R. (1996). Arachidonic and docosahexaenoic acids are biosynthesized from their 18-carbon precursors in human infants. *Proc. Natl. Acad. Sci. USA*, **93**, 49–54.

Scott, B. L. and Bazan, N. G. (1989). Membrane docosahexaenoate is supplied to the developing brain and retina by the liver. *Proc. Natl. Acad. Sci. USA*, **86**, 2903–7.

Shirai, K., Saito, Y., Yoshida, S. and Matsuoka, N. (1986). Existence of lipoprotein lipase in rat brain microvessels. *Tohoku J. Exp. Med.*, **149**, 449–50.

Tayarani, I., Chaudiere, J., Lefauconnier, J. M. and Bourre, J. M. (1987). Enzymatic protection against peroxidative damage in isolated brain capillaries. *J. Neurochem.*, **48**, 1399–402.

Tayarani, I., Cloez, I., Clément, M. and Bourre, J. M. (1989). Antioxidant enzymes and related trace elements in aging brain capillaries and choroid plexus. *J. Neurochem.*, **53**, 817–24.

Ziboh, V. A. and Chapkin, R. S. (1988). Metabolism and function of skin lipids. *Prog. Lipid Res.*, **27**, 81–105.

Ziylan, Z. Y., Bernard, G. C., Lefauconnier, J. M. *et al.* (1992). Effect of dietary n-3 fatty acid deficiency on blood-to-brain transfer of sucrose, -aminoisobutyric acid and phenylalanine in the rat. *Neurosci. Lett.*, **137**, 9–13.