Astrocytes are mainly responsible for the polyunsaturated fatty acid enrichment in blood–brain barrier endothelial cells in vitro

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Abstract To determine the respective roles of endothelial cells from brain capillaries and astrocytes in the conversion of circulating 18:2n–6 and 18:3n–3 into 20:4n–6 and 22:6n–3, respectively, a coculture of the two cell types mimicking the in vivo blood–brain barrier was used. During the culture period, endothelial cells cultured on an insert were set above the medium of a Petri dish containing or not a stabilized culture of astrocytes. Five days after confluence, labeled 18:2n–6 and 18:3n–3 (10 μm each) were added to the endothelial cells and incubated for 48 h. Analogous experiments were also performed by using each cell type cultured alone in the culture device. The distribution of radioactivity in lipids and fatty acids was studied in all the compartments of the culture device. Endothelial cells cultured alone weakly converted the precursor fatty acids into 20:4n–6 and 22:6n–3. When endothelial cells were cocultured with astrocytes, their content of polyunsaturated fatty acids increased dramatically. This effect was associated with the uptake of polyunsaturated fatty acids from the lower medium (astrocyte medium). These fatty acids were released by astrocytes after they were synthesized from the precursor fatty acids that passed through the endothelial cell monolayer into the lower medium. Polyunsaturated fatty acids were released by astrocytes as unesterified fatty acids and as phospholipids (mainly phosphatidylcholine and lysophosphatidylcholine) even when the medium was devoid of serum. These results suggest that astrocytes could play a major role in the delivery of essential polyunsaturated fatty acids to the barrier itself and to the brain.—Bernoud, N., L. Fenart, C. Bénistant, J. F. Pageaux, M. P. Dehouck, P. Molière, M. Lagarde, R. Cecchelli, and J. Lecerf. **Astrocytes are mainly responsible for the polyunsaturated fatty acid enrichment in blood–brain barrier endothelial cells in vitro. *J. Lipid Res.* 1998.** 39: **1816–1824.**

Supplementary key words blood–brain barrier model • coculture • free fatty acids • phosphatidylcholine • lysophosphatidylcholine • lipid release

The brain contains high proportions of polyunsaturated fatty acids such as arachidonic (20:4n–6) and docosahexaenoic (22:6n–3) acids but only small proportions of their respective precursors, linoleic (18:2n–6) and linolenic (18:3n–3) acids (1–4). Whereas the presence of 20:4n–6 can be easily explained by the functional importance of eicosanoids, that of 22:6n–3 is less understood although it is now established that impaired levels of this fatty acid are responsible for disturbances in visual acuity, behavior, and learning (5–7). However, the mechanisms responsible for the selective accumulation of both fatty acids in the brain are still unknown. The blood–brain barrier (BBB) could play a major role by controlling the passage of polyunsaturated fatty acids or by converting 18:2n–6 and 18:3n–3 from the blood into 20:4n–6 and 22:6n–3, respectively (8, 9). Indeed, endothelial cells from the brain microvessels are rich in 20:4n–6 and 22:6n–3 (10, 11). In a previous study (12) we showed that capillary endothelial cells cocultured with astrocytes in a BBB model were enriched in 20:4n–6 and 22:6n–3, as compared to endothelial cells cultured without astrocytes. This raises the question of the ability of capillary endothelial cells to convert the two essential fatty acids and the question of the type of cooperation between the two cell types. Are the capillary endothelial cells of the reconstituted BBB able to synthesize 20:4n–6 and 22:6n–3 from their 18 carbon precursors, as it has been recently reported in cultures of microvessels from bovine retina and rat brain (13), or are they weakly capable (9) or unable to convert 22:5n–3 into 22:6n–3 as shown in cultures of different lines of murine cerebromicrovascular endothelial cells (14)? Is the presence of astrocytes in the surrounding area able to stimulate biosyntheses in endothelial cells or must the astrocytes first convert

Abbreviations: BBB, blood–brain barrier; PL, phospholipids; FFA, free fatty acids; PC, phosphatidylcholine; LPC, lysophosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol.

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22:5n–3 into 22:6n–3 as is the case for 22:6n–3 accretion in neurons (15)? To answer these questions we have used a coculture model (12) and studied the conversion of labeled 18:2n–6 and 18:3n–3 by brain capillary endothelial cells and astrocytes cultured alone or cocultured in the BBB model. We found that endothelial cells from bovine brain capillaries weakly synthesized 20:4n–6 and 22:6n–3 but mainly took up these fatty acids synthesized by cocultured astrocytes and released into the medium in free form or esterified in phospholipids (PL).

MATERIALS AND METHODS

Chemicals

Linoleic and linolenic acids and the main reference fatty acids for metabolic conversion studies were obtained from Fluka (Switzerland). Standard 24:5n–3 and 24:6n–3 were kindly provided by Dr. H. Sprecher, Ohio State University). [1-^{14}C]linoleic acid (53 mCi/mmol) and [1-^{14}C]linolenic acid (52 mCi/mmol) were purchased from NEN Du Pont, France. Bovine serum albumin was obtained from Sigma (St. Louis, MO).

Cell cultures

Culture of isolated capillary endothelial cells from bovine brain was performed as described by Méresse et al. (16), and culture of isolated rat astrocytes and the cocultures of the two cell types were performed as detailed in our previous work (12). The culture device is briefly described herein (**Fig. 1**). Endothelial cells (used between passages 4 and 7) were cultured to confluence on the plate of an insert constituted by a membrane (Transwell polycarbonate, pores of 0.4 μm) coated on the upper side by the rat tail collagen. During the entire culture period, the insert was set over the medium of a Petri dish with or without stabilized astrocyte cultures. This device allows molecules crossing the endothelial cell monolayer to be recovered in the lower medium and in astrocytes when they are present. When endothelial cells are cocultured with astrocytes their monolayer exhibits some of the characteristics of the BBB (17).

Incubations with labeled linoleic and linolenic acids

Labeled linoleate and linolenate were bound to bovine serum albumin (2 mol fatty acid per mol albumin) in Dulbecco's modified Eagle medium (DMEM) containing albumin. Fifty μL of solution containing 15 nmol of each fatty acid (0.78 μCi of 18:2n–6 and 0.795 μCi of 18:3n–3) was added to the endothelial cell medium (5 days after confluence) or in some cases to the astrocyte medium. In all cases the concentration of each fatty acid in the medium was 10 μm. Incubations were performed for 48 h at 37°C. At the end of incubations, media were removed, cells were rinsed twice with phosphate-buffered saline and the washings were added to the corresponding incubation medium. Finally, cells were scraped off and the insert membranes were removed. The different media were centrifuged at 5000 *g* for 15 min to remove cell fragments.

To determine the irreversible adsorption of lipids on the dish or insert walls, control incubations were performed by incubating labeled fatty acids bound to albumin in the device containing incubating medium but without cultured cells. The radioactivity loss was measured and the value (15.1% ± 0.5) was subtracted for calculations of fatty acid oxidation.

Statistical analyses

The two-tailed unpaired *t* test was used to compare the data from cocultures and cultures of isolated cells.

Lipid and fatty acid analyses

Lipids from media were extracted by the Bligh and Dyer method (18) and those from cells and insert membranes were extracted according to the method of Folch, Lees, and Sloane Stanley (19). The radioactivity of each lipid extract and each aqueous phase from extraction was determined on aliquots. Lipids were separated by thin-layer chromatography on silica gel 60 plates. Neutral lipid classes and total PL were separated using the solvent system hexane–diethyl ether–acetic acid 70:30:1 (vol/vol) and PL classes were separated using either the solvent mixture chloroform–methanol–methylamine 60:20:5 (vol/vol) or chloroform–methanol–water 65:25:4 (vol/vol). The radioactivity of lipid bands was determined by radioscanning (Berthold, Silena). Each band was scraped off and extracted with chloroform–methanol 2:1 (vol/vol). In some cases, PL classes were separated by high performance liquid chromatography (HPLC) in isocratic

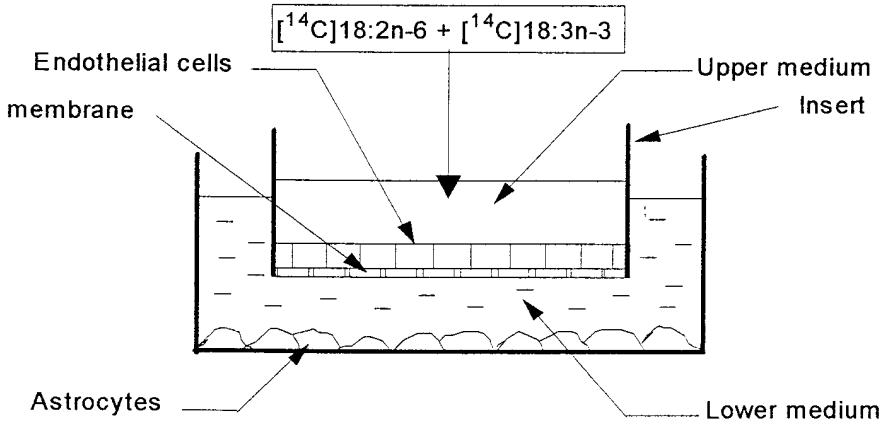

Fig. 1. Coculture device. Endothelial cells from bovine brain were cultured until confluence on an insert set over the medium of the stabilized culture of rat astrocytes. The culture medium was DMEM containing 10% horse serum and 10% calf serum. Four to 5 days after confluence, labeled fatty acids were incubated in the upper medium for 48 h. In some cocultures, fatty acids were incubated in the lower medium. Several incubations were also performed by using cocultures with a lower medium devoid of serum for 24 h. The same culture device was also used for culture of each cell type alone.

conditions according to the method described by Bernhard et al. (20) and lipids were collected from the outlet of the UV detector. Isolated lipid classes were transmethylated using methanol–5% sulfuric acid (vol/vol) at 90°C for 2 h. The resulting fatty acid methyl esters were separated by HPLC as described by Pageaux et al. (21) with the following slight modification: the initial mobile phase composition was acetonitrile–water 77:23 (vol/vol) and was programmed to reach 90:10 (vol/vol) after 1 h. The methyl ester radioactivity was measured either by direct flow scintillation counting or by usual liquid scintillation counting after peak collection. Each HPLC peak was identified by using references and by analysis of collected peaks by gas chromatography–mass spectrometry. In the HPLC conditions used, all the methyl esters of each n–6 and n–3 fatty acid were separated except for the two 18:3 acids. Because the radioactivity of 18:3n–6 (as determined in other conditions) was always very low and negligible by comparison with that of 18:3n–3, it was possible to determine the radioactivity of each fatty acid from each family when 18:2n–6 and 18:3n–3 were incubated together.

RESULTS

Distribution of radioactivity within the compartments of coculture

In the coculture conditions, the n–3/n–6 fatty acid radioactivity ratio of the upper medium after 48 h of incubation was close to that of the two parent fatty acids at the start of incubation (**Fig. 2**). However, more radioactivity from 18:2n–6 than from 18:3n–3 was recovered in the lower medium, endothelial cells, and astrocytes. Conversely and reciprocally, n–6 fatty acids were less oxidized than n–3 fatty acids (oxidation was estimated as the sum of radioactivity in aqueous phases from lipid extractions and radioactivity lost during the incubation). When 18:2n–6 or 18:3n–3 were added separately (not shown) to the coculture, the distribution of radioactivity was analogous to that observed when fatty acids were incubated together, but the incorporation of each fatty acid in the two cell types was enhanced and the oxidation of n–6 fatty acids was lowered.

Influence of coculture on the metabolic conversions of precursor fatty acids

The distribution of radioactivity within the fatty acids of cell phospholipids (more than 95% of the cell lipid radioactivity in both cell types) showed that endothelial cells cultured alone weakly converted 18:2n–6 and 18:3n–3 into long chain polyunsaturated fatty acids **(Fig. 3**, upper panel hatched bars). Figure 3 shows that 77.7 and 17.7% of the total PL radioactivity was recovered in 18:2n–6 and 18:3n–3, respectively. However, calculations showed that 18:2n–6 and 18:3n–3 accounted for 98.4 and 84.0% of total n–6 and total n–3 labeled fatty acids, respectively. Noticeable radioactivity was found in the direct elongated products of precursors, 20:2n–6 and 20:3n–3. In contrast, the radioactivity in most unsaturated products such as 20:4n–6, 22:6n–3, and 24:6n–3 was very low. Conversely, when endothelial cells were cocultured with astrocytes, the proportions of radioactivity recovered in the different polyunsaturated fatty acids from endothelial cells markedly increased (about 5-fold for 22:6n–3 and 13-fold for 20:4n–6) (Fig. 3, upper panel open bars). When endothelial cells were cocultured with astrocytes and the astrocytes were removed at the beginning of incubation (not shown), the radioactivity distribution within fatty acids in endothelial cells was analogous to that observed when incubations were performed with endothelial cells cultured alone. This indicates that the enhancement of long chain polyunsaturated fatty acid labeling from n–6 and n–3 fatty acid precursors in endothelial cells was dependent on the presence of astrocytes and their medium. On the other hand, astrocytes cultured alone actively converted 18:2n–6 and 18:3n–3 into long chain derivatives (Fig. 3, lower panel hatched bars). 20:4n–6 and 22:5n–3 were the main n–6 and n–3 fatty acids synthesized while 22:6n–3 and 20:5n–3 were also produced to a lesser extent. Small proportions of radioactivity were always present in 24:5n–3 and 24:6n–3. When astrocytes were cocultured with endothelial cells, the proportions of radioactivity in 20:4n–6, 22:6n–3,

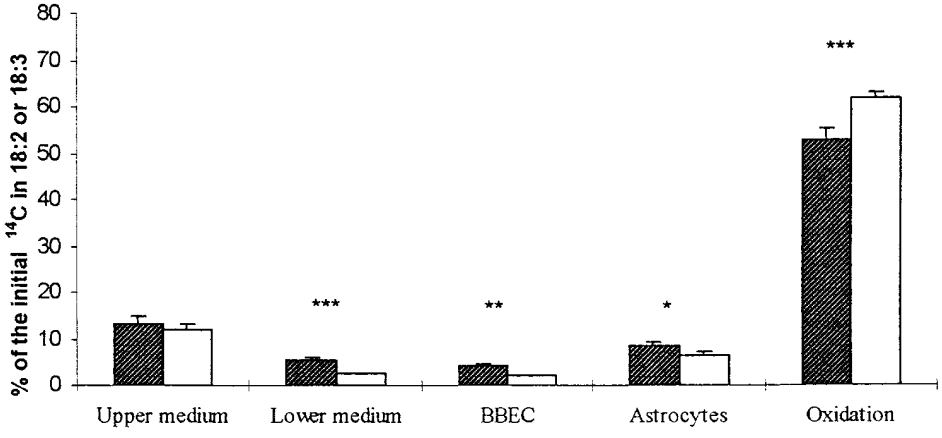

Fig. 2. Distribution of radioactivity within the compartments of the coculture 48 h after incubation with [^{14}C]18:2n–6 and [^{14}C]18:3n–3. The culture and incubation conditions are described in Fig. 1. Hatched bars: n–6 fatty acid radioactivity, open bars: n–3 fatty acid radioactivity. Comparison of n–6 versus n–3 fatty acid radioactivity *,**,***: $P < 0.05$, 0.01, and 0.001, respectively. Results are means ± SD of 9 values (3 independent incubations from 3 batches).

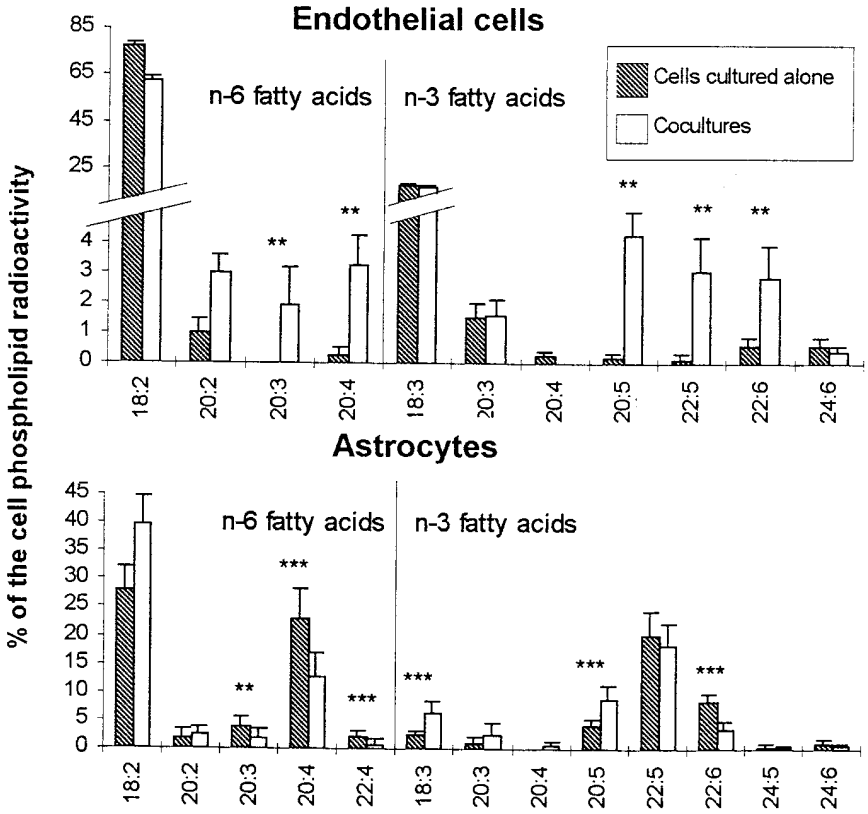

Fig. 3. Radioactive fatty acid distribution within PL from endothelial cells and astrocytes. The culture and incubation conditions are described in Fig. 1. Comparison of cells alone versus cocultures; *,**,***: $P < 0.05$, 0.01, and 0.001, respectively. Results are means ± SD of 9 values (3 independent incubations from 3 batches).

20:3n–6, and 22:4n–6 were decreased and that in 20:5n–3 and the two parent fatty acids was enhanced (Fig. 3, lower panel open bars). With the exception of 20:5n–3 this was the opposite of what was observed in endothelial cells. Altogether these results reinforce the idea that astrocytes play a role in the polyunsaturated fatty acid content of endothelial cells in coculture. Therefore we studied the distribution and location of radioactive fatty acids in the lower medium as an index of fatty acid release by astrocytes.

Lipid radioactivity of endothelial cell and astrocyte media

When astrocytes were either cultured alone or cocultured, lipid radioactivity from the lower medium was mainly recovered in the free fatty acid fraction (FFA) and in PL (**Fig. 4**, A–C), the remaining radioactivity being essentially attached to what chromatographed as hydroxy fatty acids (not shown). When endothelial cells were cultured alone (Fig. 4, D), more than 98.7% of lipid radioactivity from the lower medium was found in FFA. When astrocytes were cultured alone (Fig. 4, A), 58.2% of medium lipid radioactivity was in PL. The proportion recovered in PL fell to 15.5% in coculture (Fig. 4, C), but was 31.1% when 18:2n–6 and 18:3n–3 were added to the astrocyte medium of coculture instead of the endothelial cell medium (Fig. 4, B). As mentioned at the bar tops of Fig. 4, a significant percentage of the initial radioactivity was recovered in the medium PL. As a matter of fact, when astrocytes were cultured alone, PL radioactivity represented 1.28% of the initial radioactivity while that of astrocyte lipids represented less than 10% (not shown). Altogether these results suggest that astrocytes were responsible for PL radioactivity in the astrocyte medium of cocultures.

By comparison, more than 95% of lipid radioactivity in the upper medium was in FFA whatever the conditions used, the rest being associated with compounds that migrated as hydroxy fatty acids and the origin of the TLC separation lane (not shown).

In cocultures, roughly two thirds of PL radioactivity from the lower medium was recovered in phosphatidylcholine (PC) and one third in lysophosphatidylcholine (LPC), (**Fig. 5**, upper panel I a–b). This was true when astrocytes were either cultured alone (Fig. 5, upper panel I a), or cocultured for 48 h (Fig. 5, upper panel I b). Some radioactivity was recovered to a lesser extent in phosphatidylethanolamine, especially when 18:3n–3 was incubated alone (not shown). Only traces of radioactivity were observed in phosphatidylinositol and phosphatidylserine. The culture media used for culture of astrocytes and endothelial cells contained 10% horse serum and 10% calf serum. Therefore it could be assumed that the presence of labeled PL in the astrocyte medium would be due to the presence of acceptors such as lipoproteins. In order to check this possibility, incubations of both fatty acids were performed in cocultures by using an astrocyte medium

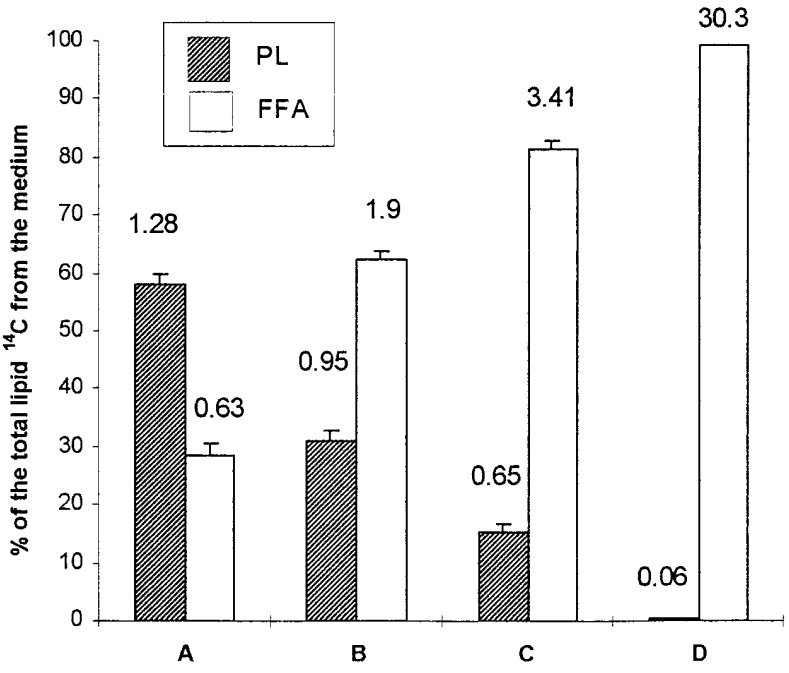

Fig. 4. Influence of the culture type and fatty acid availability to cells on the total radioactivity in phospholipids and free fatty acids in the lower medium. A: Astrocytes cultured alone; B: Incubations with 18:2n–6 and 18:3n–3 in the lower medium of cocultures; C: Incubations with 18:2n–6 and 18:3n–3 in the upper medium of cocultures; D: Endothelial cells cultured alone. The culture and incubation conditions are described in Fig. 1. The numbers at the bar tops are % of the incubated radioactivity. Results are means ± SD of 3 independent incubations.

without serum during the incubation period. The incubation period was reduced to 24 h to prevent metabolic deficiencies in cells. In the presence of serum for 24 h, the radioactivity distribution within PL of the lower medium (Fig. 5, upper panel II c) was analogous to that observed after 48 h although PL radioactivity of the astrocyte medium was lower. A similar distribution was observed in cocultures without serum (Fig. 5, upper panel II d), but PL supported a higher proportion of radioactivity from the lower medium lipids than that observed in the presence of serum (Fig. 5, upper panel II c). Therefore, the PL radioactivity (mainly in PC and LPC) in the astrocyte medium was not linked to the presence of serum in the medium.

The radioactivity distribution within PL classes from the lower medium was quite different from that of the endothelial cell and astrocyte PL (95% of the radioactivity of total lipids for the two cell types) observed after 24 h (Fig. 5, lower panel). As a matter of fact, only traces of radioactivity were observed in LPC within the cellular PL. On the other hand, we have tested the possible hydrolysis in the absence of cells of low quantities of 16:0–[^{3}H]22:6–PC and 2×[^{3}H]16:0 PC incubated for 48 h in the incubation medium without serum at 37°C. The results showed that no significant hydrolysis occurred in these conditions making it unlikely that LPC recovered in the astrocyte medium resulted from a spontaneous hydrolysis of PC in the medium.

Figure 6 shows that astrocytes cultured alone or cocultured released n–3 and n–6 polyunsaturated fatty acids in their culture medium. The radioactivity distribution within fatty acids from the astrocyte medium (Fig. 6) did not reflect that observed in astrocyte PL (Fig. 3, lower panel) as, for example, 22:5n–3 did not contain the major part of radioactivity associated with n–3 fatty acids derived from 18:3n–3. When astrocytes were cocultured (Fig. 6, open bars) proportions of radioactive n–3 and n–6 polyunsaturated fatty acids from the astrocyte medium decreased (except that of 20:5n–3 which increased) in comparison with astrocytes cultured alone (Fig. 6, hatched bars). It is noteworthy that the radioactivity proportions of these fatty acids markedly increased in the endothelial cells in cocultures. These results show that the release of polyunsaturated fatty acids was controlled by astrocytes and was different according to the coculture conditions.

In **Fig. 7** (panels A–B) we show the radioactivity distribution within fatty acids in the PL and FFA fractions from the lower medium after coculture and incubation for 24 h using an astrocyte medium devoid or not of serum. The figure shows that in the absence of serum (Fig. 7, panel A), polyunsaturated fatty acids were present approximately in the same proportions in the two lipid forms with a small preference for the unesterified form. The addition of serum (Fig. 7, panel B) induced no change in the polyunsaturated fatty acid content of PL from the astrocyte medium but enhanced the proportion of the fatty acid recovered in the unesterified fraction.

DISCUSSION

Moore, Yoder, and Spector (14) have reported that endothelial cells from several lines of murine cerebromicrovessels are able to synthesize polyunsaturated fatty acids from 18:2n–6 to 22:4n–6 and 18:3n–3 to 22:5n–3 but are not able to produce 22:6n–3 from 22:5n–3. Conversely, a recent study shows that endothelial cells from rat brain microvessels and bovine retinal microvessels actively synthesize 22:6n–3 (13). We show in the present study that endothelial cells from bovine brain capillaries cultured alone were able to form 22:6n–3 and 24:6n–3 only in small

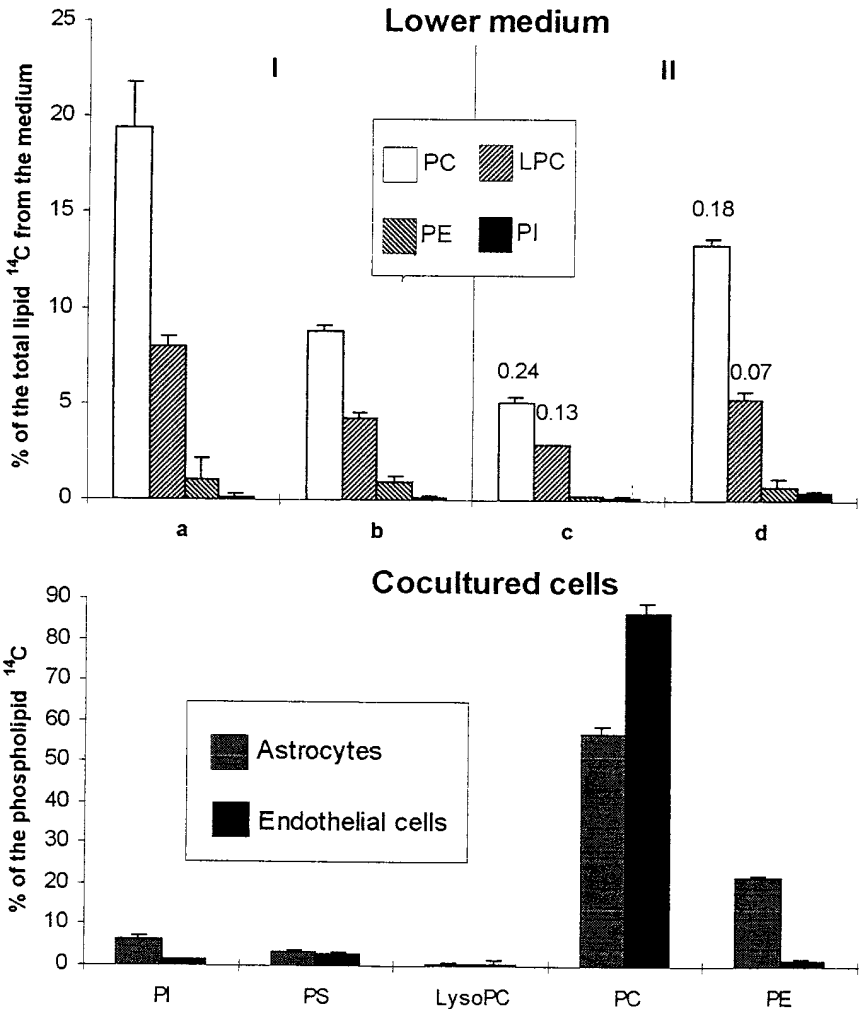

Fig. 5. Distribution of radioactivity within the phospholipid classes from the lower medium lipids according to the culture conditions. Comparison with the distribution observed in cellular phospholipids. Upper panel Part I: Incubations with serum in the lower medium for 48 h, a: cultured astrocytes alone; b: cocultures. Part II: Incubations for 24 h, c: cocultures with serum in the lower medium; d: cocultures without serum in the lower medium. Lower panel: Radioactivity of the phospholipid classes from cocultured cells after 24 h incubation with serum. The numbers at the bar tops are % of the incubated radioactivity. The culture and incubation conditions are reported in Fig. 1. Results are means ± SD of 3 independent incubations.

proportions. Our results, therefore, appear as intermediary between the two above-mentioned reports and close to a more recent report of Moore (9) who showed a weak synthesis of 22:6n–3 by rat cerebromicrovessels. However, differences between our results and those of Delton-Vandenbroucke, Grammas, and Anderson (13) likely result from utilization of a more proximal precursor (22:5n–3) by those authors and, therefore, a lesser dilution of radioactivity by intermediates than in our study. In our reconstituted BBB conditions, the increase in polyunsaturated fatty acid radioactivity of endothelial cells was due to the conversion of the two parent fatty acids by astrocytes. This seems to agree with suggestions of Moore (8, 9) for a cellular cooperation at the BBB level. Our results also suggest that the previously reported changes (12) in the profile of polyunsaturated fatty acids within endothelial cells induced by the cocultured astrocytes were due to the biosynthesis and release of polyunsaturated fatty acids by astrocytes.

As has recently been reported in cultured brain and retinal endothelial cells (13), the presence of radioactivity in 24:5n–3 and 24:6n–3 in the endothelial cell monolayer and astrocytes suggests that, in both cell types, 22:6n–3 was synthesized by elongation of 22:5, desaturation, and β-oxidation of 24:6 (22–24)—likely in peroxisomes (24)—although existence of this pathway was recently questioned (25). However, the radioactivity accumulation in 22:5n–3 observed in astrocytes, and the weak synthesis of 22:6n–3 by endothelial cells alone, suggest that either 22:5n–3 elongation–desaturation or peroxisome steps of the process would be limiting factors for the 22:6 production.

Endothelial cells allowed the incubated fatty acids to cross through their monolayer as in both cultured and cocultured endothelial cells 18:2n–6 and 18:3n–3 were the main fatty acids recovered in the lower (or astrocyte) medium. Such a passage has already been described in different culture conditions (8, 9). In endothelial cells cultured

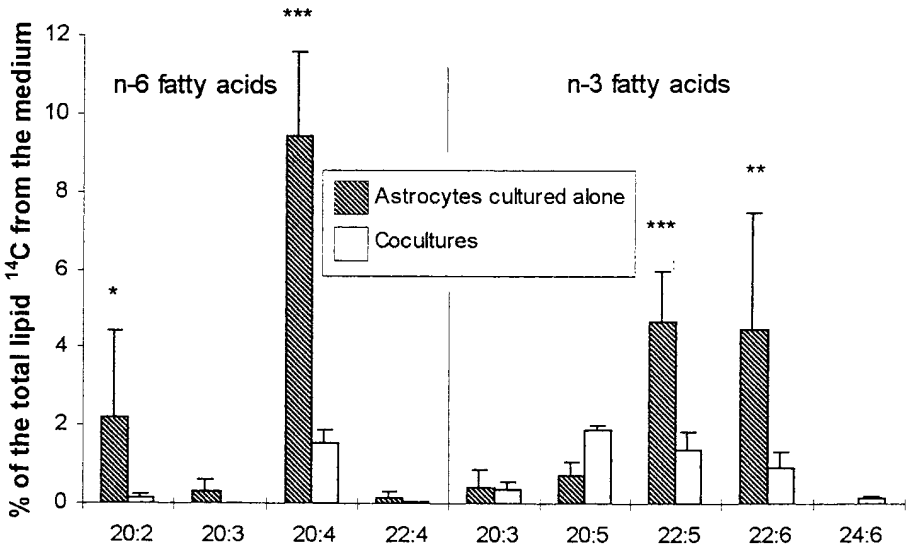

Fig. 6. Influence of the coculture on the radioactivity distribution within the polyunsaturated fatty acids recovered in the lower medium lipids. Comparison of astrocytes cultured alone versus coculture; *,**,***: $P <$ 0.05, 0.01, and 0.001, respectively. Results are means ± SD of 9 values (3 independent incubations from 3 batches).

alone, only unesterified parent fatty acids were recovered in the lower medium whereas in cocultures polyunsaturated fatty acids synthesized by astrocytes from the parent fatty acids appeared in the lower medium. The presence of unesterified polyunsaturated fatty acids in the medium of capillary endothelial cells (8, 9, 13) or astrocytes (8, 9) cultured alone has also been reported but little information is available on cocultures mimicking the blood–brain

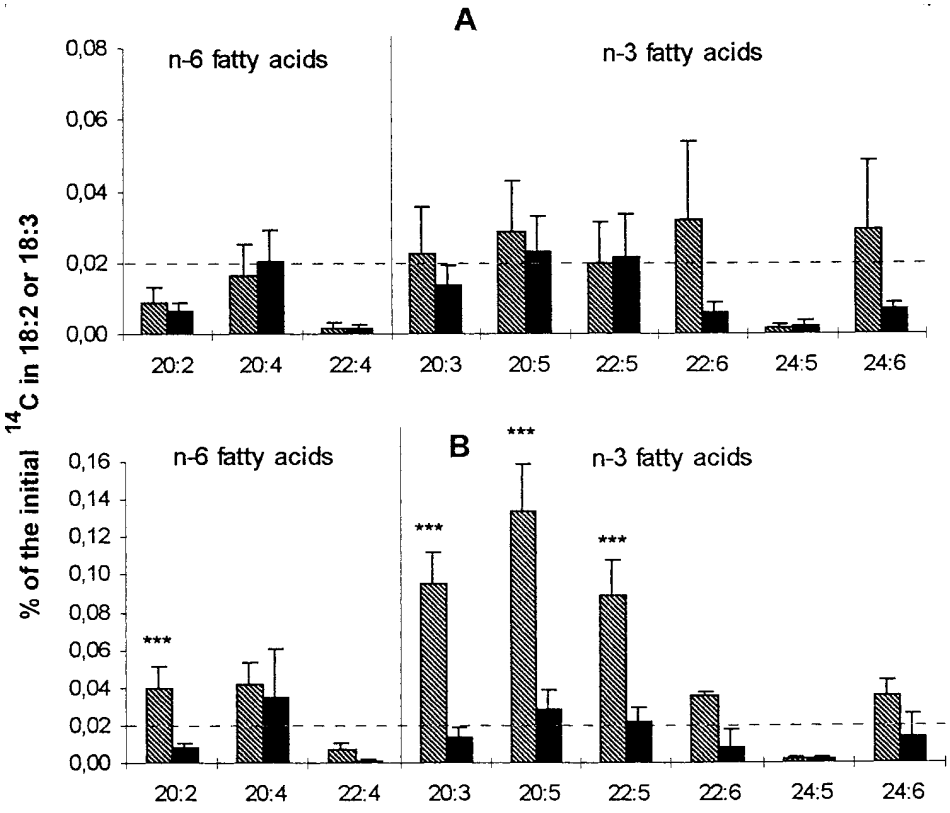

Fig. 7. Influence of serum on the distribution of long-chain polyunsaturated fatty acids in the lower medium free fatty acids and phospholipids after 24 h incubations of cocultures. Panel A: Cocultures without serum in the lower medium, panel B: cocultures with serum in the lower medium. Hatched bars: free fatty acids; black bars: phospholipids. Comparison of incubations with serum versus without serum; *,**,***: $P <$ 0.05, 0.01, and 0.001, respectively. Results are means ± SD of 3 independent incubations.

barrier. Moore (8, 9) has reported the presence of 22:6n–3 in the lower medium of cocultures after 96 h without specifying either its lipid form or the existence of accompanying polyunsaturated fatty acids. Our results show that polyunsaturated fatty acids were also released as PL by astrocytes, which raises the question of the release modalities. When the astrocyte medium of cocultures did not contain any serum, polyunsaturated fatty acids were distributed about half-and-half between the PL and the unesterified forms. When serum was added to the astrocyte medium, only the unesterified form was enhanced. This suggests that in classical culture conditions, the level of released polyunsaturated fatty acids is mainly dependent on the presence of serum, likely because serum contains lipid carriers such as albumin and lipoproteins. In physiological conditions, the composition of the intercellular space fluid between endothelial cells and astocytes is likely to be different as serum albumin and lipoproteins normally do not cross the barrier in noticeable proportions. However, using the present BBB model, we have shown the existence of an LDL transcytosis across endothelial cells (26) via the LDL receptor (27), which is regulated by the nutritional state of astrocytes. There are several lymphatic systems in the brain (28), but unfortunately no information is available on their fluid composition except for systems in relation with the cerebrospinal fluid (CSF) of which the composition is known. CSF contains very low levels of albumin and lipoproteins of different composition compared to that of serum (29–32). This suggests that special lipoproteins would be secreted in the brain as proposed by Pitas et al.(29). Moreover, it is known that astrocytes are able to synthesize apolipoproteins (29, 33–35). As polyunsaturated fatty acids were released in the unesterified and PL forms by astrocytes in a medium devoid of serum, it may be suggested that astrocytes released the carriers of the two lipid forms. However, it is also possible that astrocytes facilitate the passage of these carriers across the endothelial cells as we have reported for LDL (26). If the presence of PC in putative lipoprotein-like carriers is not unlikely, a high proportion of LPC is unexpected. This observation might be related to the observation that LPC is a good carrier for polyunsaturated fatty acids in vivo (36, 37), especially for their delivery to the developing brain (38).

In conclusion, our work shows that endothelial cells from brain capillaries cocultured with astrocytes in a system that mimics the BBB were able to produce 20:4n–6 and 22:6n–3 from 18:2n–6 and 18:3n–3, respectively. However, the 20:4 and 22:6 content of endothelial cells was mainly dependent on synthesis in astrocytes and release of polyunsaturated fatty acids either in the unesterified form or esterified in PL (mainly PC and LPC). The significance of these PL classes and that of the release of putative carriers by astrocytes should be addressed in further studies.

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