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1-Acyl-2-lysophosphatidylcholine transport across the blood–retina and blood–brain barrier

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Abstract

The transport of lysophospholipids through the rat blood–retina and blood–brain barrier was determined by using radioactive 1-palmitoyl-2-lysophosphatidylcholine (Pam-lysoPtdCho) and by measuring the uptake of this labeled compound into the retina and various brain regions after short in situ carotid perfusion. The transport was not affected by probenecid (0.25 mM), but it was inhibited, in a dose-dependent manner, by circulating albumin which is able to bind tightly to lysophosphatidylcholine and lowered the availability of the latter for tissue extraction. Radiotracer transfer in the retina was higher than in brain regions. The permeability-surface area products (*PS*) changed with the inclusion of unlabeled Pam-lysoPtdCho, showing that transport across retinal and brain microvessels is mainly saturable. The data provided an estimate of transport constants (V_{max}, K_m and non-saturable constant K_d). However, we could not distinguish whether this saturable process represents the saturation of a transport carrier or simple passive diffusion followed by the saturation of enzymatic reactions. In brain tissue lipid extract, 20 s after carotid injection, radiolabel was associated by 45% to unmetabolized Pam-lysoPtdCho. Partial acylation to phosphatidylcholine, as well as hydrolysis and redistribution of the fatty acyl moiety into main phospholipid classes also occurred. The present results, compared to our previous data, indicate that Pam-lysoPtdCho is transported faster and/or in greater amounts than unesterified fatty acids.

Key words: Lysophosphatidylcholine; Fatty acid; Blood–brain barrier; Retina; Lipid metabolism

1. Introduction

Lysophosphatidylcholine (lysoPtdCho) is a normal constituent of mammalian plasma representing 5–20% of total phospholipids. In the rat, it is the second most prevalent plasma phospholipid with a concentration of 0.20–0.25 mM [1,2]. In the blood, lysoPtdCho is complexed to albumin [3] and lipoproteins [2,4,5], and originates from two metabolic pathways: (i) the transesterification of PtdCho and free cholesterol catalyzed by an acyltransferase (LCAT), a reaction which is responsible for the more saturated lysoPtdCho species [6]; (ii) the direct secretion of more unsaturated species by the liver [7–9]. In this organ, lysoPtdCho arises from the deacylation of PtdCho by phospholipases A_1 and A_2. Plasma lysoPtdCho has been considered a transport form to tissues, and especially the brain, of both choline and unsaturated fatty acids [2,10–12].

The possibility of lysoPtdCho transport into the brain is strongly suggested by several incorporation experiments. Labeled lysoPtdCho injected into the blood stream rapidly disappeared from the circulation and is mainly recovered in: (a) PtdCho isolated from several organs [13], including the brain [11]; (b) fatty acid fraction because of hydrolysis catalyzed by tissue lysophospholipase activity, or (c) water-soluble degradation intermediates (choline, P-choline and betaine) because of extensive enzymatic fragmentation [11]. The uptake of intact, albumin-bound saturated and unsaturated 1-acyl or 2-acyl-lysoPtdCho by developing rat brain after intravenous perfusion per 30 s has been demonstrated [2]. It is well known that both species turn over in the brain even if 2-acyl-lysoPtdCho is likely to be reacylated to form PtdCho more actively than the 1-acyl species [14]. Unsaturated 2-acyl-lysoPtdCho is taken up by total brain more efficiently than saturated ones or the corresponding unesterified fatty acids, even if the metabolic fate of the liberated fatty acid in the brain is not exactly the same. However, Pardridge and co-workers [15] have questioned whether lysoPtdCho is able to cross the blood–brain barrier. The present experiments were performed to investigate whether: (i) 1-palmitoyl-lysoPtdCho (Pam-lysoPtdCho) could be transported across the blood–brain barrier (BBB) and blood–retina barrier (BRB); (ii) fatty acids esterified to lysophospholipids are transported more or less efficiently than their unesterified form.

2. Materials and methods

The uptake of 1-acyl-L-1-([1-^{14}C]palmitoyl)-2-lyso-*sn*-glycero-3-phosphocholine across the capillaries of the retina, optic nerve, intracranial structures of the visual system (lateral geniculates, superior colliculi, visual cortex) and other main brain CNS areas was measured in groups of Sprague–Dawley male rats aged 4 months. The in situ brain perfusion technique of Takasato et al. [16] was used as in our previous studies [17,18], except that fatty acid-free bovine serum albumin (BSA, at the concentrations 0–1.0 mM) and no Ca^{2+} ions were

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Abbreviations: *PS*, permeability-surface area product; lysoPtdCho, lysophosphatidylcholine; Pam-lysoPtdCho, 1-palmitoyl-2-lysophosphatidylcholine; PtdCho, phosphatidylcholine; BRB, blood–retina barrier; BBB, blood–brain barrier.

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included in bicarbonate-based salt solution (HBSS). The medium, containing 142.0 NaCl, 28.0 $NaHCO_3$, 4.2 KH_2PO_4, 1.0 $MgSO_4$ in μmol/ml and 6 μmol/ml glucose, was equilibrated with a gas mixture O_2/CO_2 (95:5%) at 37°C for 5 min. The pH was checked and equaled 7.4. Radioactive 1-acyl-lysoPtdCho was dissolved in HBSS to give a 20 μM concentration in the perfusate, with a specific radioactivity of 56 mCi/mmol (Amersham Int. plc, England). In a set of experiments, unlabeled 1-palmitoyl-lysoPtdCho (final concentration, 0.03–1.5 mM) was also added. In all experiments in which [^{14}C]lysophosphatidylcholine was used in the perfusion medium, [^{3}H(G)]inulin (291 mCi/g, NEN-Du Pont) was included in the perfusate (^{3}H/^{14}C ratio 5:1) in order to measure intravascular space.

In brief, the technique involves cannulating with thin-wall polyethylene catheter (PE-30) the left common carotid artery of animals anaesthetized with continuous flow of O_2/N_2O gas mixture, containing 2% halothane during the initial surgery. The left external carotid artery, rostrally to the bifurcation from the common carotid, was tied off whereas the pterygopalatine branch was left open. At zero time, halothane was discontinued and the common carotid artery was ligated; the perfusion of the internal carotid was begun at a constant rate by a perfusion pump and continued with HBSS solution for periods up to 20 s. The temperature of the injectate was 35–37°C, and the perfusion rate was 83 μl/s. During the short time of perfusion, the thorax of the animal was opened and the left ventricle of the heart was cut. At the end of the perfusion, the rats were decapitated, the eyes dissected and the brain, including the optic nerves, was removed from the skull and washed with ice-cold saline. The meninges and blood vessels on the surface of each brain region were carefully removed while on a chilled metal platform, and the various brain regions of the perfused side were dissected out, weighed on chilled filter paper moistened with saline and digested overnight at 50°C in 1 ml Soluene 100 (Packard, Ill.). Two hours after a bleaching process with a few drops of 35% H_2O_2, they were prepared to be assayed for radioactivity in a scintillation counter by the addition of 10 ml of Instagel (Packard, Ill.). Desheathed optic nerves and retinas were also collected from the left eye bulbs, and processed as previously described [17]. Perfusate flow (F) through the brain regions was measured using [methyl-^{3}H]diazepam (84.2 mCi/mmmol, NEN-Du Pont). The perfusion rate was 83 μl/s and the perfusion time was 7 s. F and PS were determined from the relative equations as described by Takasato et al. [16]. The same calculations have also been applied recently by us to characterize fatty acid transport across BRB and BBB of the rat [17,18]. Assuming that the flux data could be described by a Michaelis–Menten transport model, unidirectional influx J_{in} was estimated by fitting the data to the following equation: $J_{in} = F(1-e^{-PS/F})C_{pf}$, where C_{pf} is the perfusate concentration of Pam-lysoPtdCho. V_{max} and K_m constants (the maximal transport rate of the saturable component, and the half-saturation constant of the saturable component) were obtained by using an Eadie–Hofstee transformation of the J_{in} and Pam-PtdCho concentration data. K_d, the constant of non-saturable transport, was estimated by employing the equation: $PS = V_{max}/(K_m + C_{pf}) + K_d$, reported by Smith et al. [19] for amino acid brain perfusion.

3. Results

Table 1 shows the results indicating that the control (no albumin was added to the carotid perfusate) PS index of blood–to–CNS Pam-lysoPtdCho transport was higher for the optic nerve and tract, cortex, hippocampus and olfactory bulb than for other white or gray matter areas. For the other transport index, i.e. the radioactivity ratio which broadly indicates the parenchymal penetration of [^{14}C]lysoPtdCho compared to that of undiffusible [^{3}H]inulin, the highest values belong to the retina and parietal-frontal cortex. This differentiated regional profile in lysophospholipid transport partially parallels the blood perfusate flow values measured by [^{3}H]diazepam injection [1]. Table 1 also shows that, in any CNS region, Pam-lysoPtdCho uptake (radioactivity ratio and PS product) was not inhibited by probenecid. Linoleic acid also enters the brain from the blood by a probenecid-insensitive mechanism [24]. The presence of 14 μM albumin (physiological concentration in the rat plasma) in the carotid artery injection solution inhibited radioactivity ratios, bringing about significant inhibition of PS

Table 1
Retinal and cerebrovascular uptake ratios and capillary permeability-surface area (PS) products for 1-acyl-L-1-([1-^{14}C]palmitoyl]-2-lysoPtdCho in nervous system regions of rats

Regions	Ratios[a]			PS ($s^{-1}\cdot10^4$)		
	Controls (no BSA)	Probenecid (0.25 mM, no BSA)	14 μM BSA	Controls (no BSA)	Probenecid (0.25 mM, no BSA)	14 μM BSA
Retina	44.9 ± 8.3	38.8 ± 7.3*	35.0 ± 8.4	28.8 ± 6.9	23.9 ± 5.5*	12.7 ± 3.7*[b]
Optic nerve	17.9 ± 3.7	15.3 ± 3.9	17.7 ± 2.9	39.7 ± 8.7	35.6 ± 5.7	12.8 ± 3.9*[b]
Optic tract	16.6 ± 1.6	15.4 ± 4.9	7.94 ± 2.0*	54.0 ± 10	60.0 ± 15	26.1 ± 8.8*[b]
Lateral geniculate	23.8 ± 3.7	25.5 ± 5.6	21.3 ± 6.4	27.7 ± 7.4	22.9 ± 6.3	25.1 ± 7.4
Superior colliculus	20.0 ± 4.9	24.1 ± 3.4	19.9 ± 5.3	25.2 ± 5.4	17.9 ± 4.5	23.5 ± 6.3
Visual cortex	25.8 ± 5.2	28.1 ± 8.7	21.7 ± 6.5	51.6 ± 9.9	60.0 ± 8.0	28.7 ± 8.3*[b]
Parietal cortex	44.3 ± 9.4	22.5 ± 3.4*	17.5 ± 2.6*	32.1 ± 6.3	38.5 ± 9.6	20.8 ± 7.2
Frontal cortex	49.5 ± 6.7	20.9 ± 6.5*	16.4 ± 4.3*	27.0 ± 4.0	26.0 ± 7.2	25.3 ± 6.4
Striatum	29.9 ± 4.0	30.7 ± 9.1	22.9 ± 6.0	29.3 ± 4.7	36.0 ± 9.1	12.1 ± 4.0*[b]
Hippocampus	17.5 ± 4.0	21.6 ± 6.4	17.0 ± 3.2	39.3 ± 9.2	36.1 ± 0.2	13.0 ± 0.0*[b]
Olfactory bulb	15.1 ± 2.2	17.5 ± 4.9	14.9 ± 4.7	41.8 ± 8.3	48.3 ± 9.1	10.8 ± 3.2*[b]

Data are means ± S.E.M., with $n = 3$. The PS products were determined with 20 μM 1-acyl-L-1-([1-^{14}C]palmitoyl)-2-lysoPtdCho and 20 μg/ml [^{3}H]inulin in the perfusate, at 20 s after internal carotid perfusion, using the standard assumptions and equation 9 of Takasato et al. [16]. The perfusion rate was 83 μl/s. Statistical significance of differences between probenecid or 14 μM bovine serum albumin (BSA) and control group (no BSA) was compared by Student's t-test, *$P < 0.05$. One way analysis of variance was also done, followed by Duncan's or Newman–Keuls' multiple comparison tests, using NCSS version 4.21 (Dr. J.L. Hintze, Kaysville, Utah, USA) statistical software package to identify the changes that were significantly different from control values; [b]$P < 0.05$.

[a]Each value represents the [^{14}C]lysoPtdCho/[^{3}H]inulin ratio in the tissue of a given region divided by the same ratio in the perfusate.

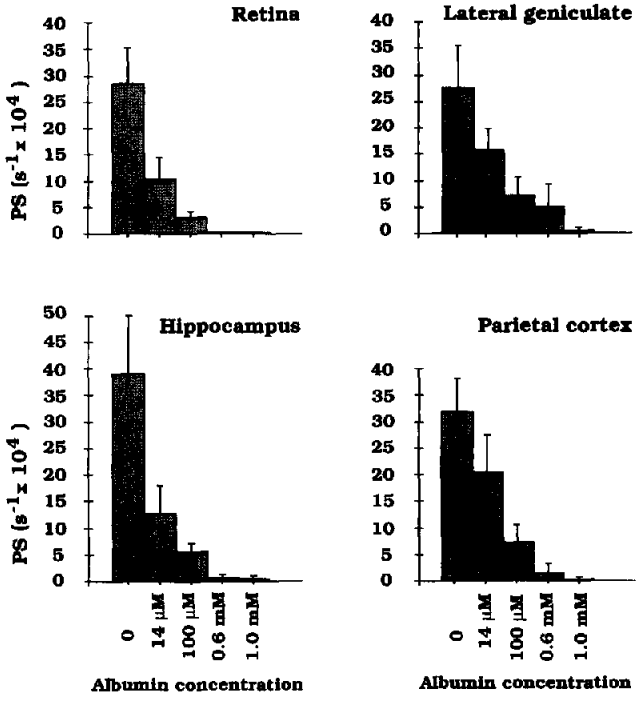

Fig. 1. Permeability-surface area product (*PS*) at 20 s after carotid injection of 1-acyl-L-1-([1-^{14}C]palmitoyl)-2-lysoPtdCho as function of free fatty acid bovine serum albumin concentration in the perfusate. Values for retina and three representative brain areas were reported. Data are mean ± S.E.M. (n = 3 rats).

products in the retina, optic nerve and tract, visual cortex and prosencephalic structures. With 5 mM Pam-lysoPtdCho in the perfusate, we did not observe any simple diffusion through the BRB and BBB.

Fig. 1 shows plots of *PS* products vs. albumin concentration for four selected CNS areas. The results for other regions (see Table 1) were quite similar. The addition of albumin to the perfusate inhibited the rate of radiotracer uptake by both the retina and brain regions. This inhibition can be explained simply by the binding of lysoPtdCho to albumin, leading to a decrease in the concentration of free lysocompound in the medium. These results are similar to those of Pardridge and Mietus [21], who found that the uptake of palmitate by the brain was a function of the fatty acid-to-albumin ratio, and hence of the concentration of the unbound fatty acid.

In all the regions studied, *PS* values decreased as a function of the increased concentration of unlabeled Pam-lysoPtdCho. With 5 mM in the perfusate, we did not observe any simple diffusion through the BRB or BBB. Fig. 2 illustrates the concentration dependence of Pam-lysoPtdCho influx into the retina and one representative brain area (parietal cortex). From the best-fit curve of J_{in} data, calculated from *PS* and C_{pf} (perfusate concentration of lysoPtdCho) values, plotted as a function of concentration, data for V_{max} and K_m were obtained. Table 2 presents mean values of the transport constants for each of the nine CNS regions examined in this study. There were marked regional differences in all three kinetic parameters. V_{max} mean values in the optic nerve and tract were two-to-three-fold greater than values in the retina and other brain regions. Among the regions, K_m varied approximately 6-fold, whereas K_d values were low (of the order of 10^{-5} ml/s/g), except for the optic nerve-tract and olfactory bulb, indicating that a non-saturable component of Pam-lysoPtdCho transport (accumulation of unmetabolized lysoPtdCho), if any, is hardly detectable.

Pooled brain areas from rats, injected for 20 s with [^{14}C]lysoPtdCho solution without or with 14 µM albumin, were extracted with chloroform/methanol (2:1, v/v),

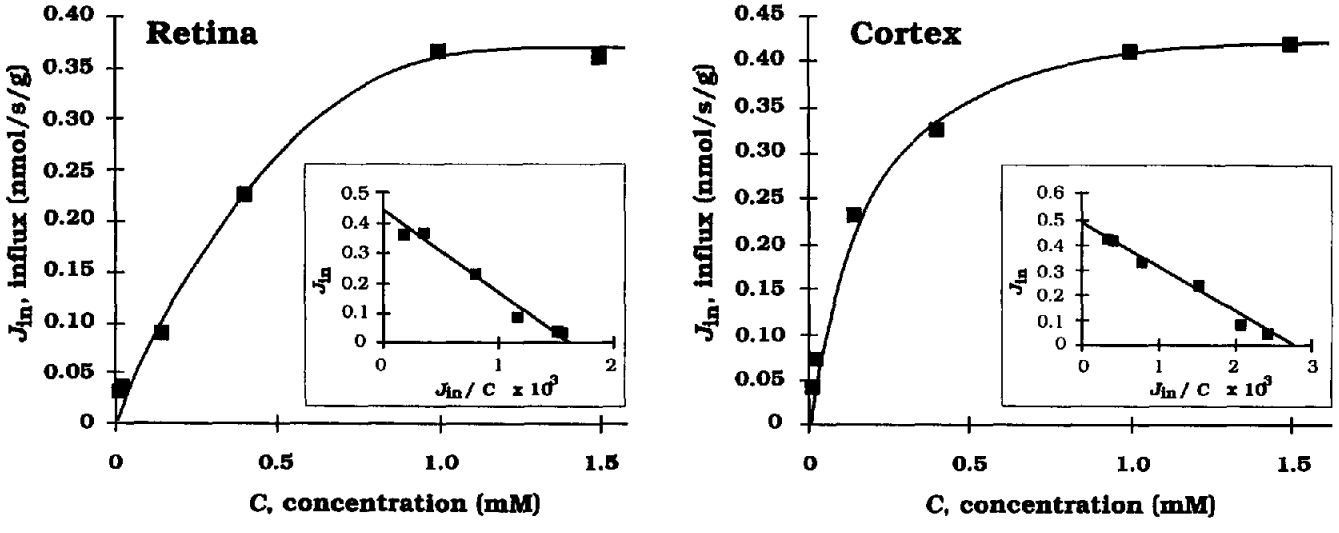

Fig. 2. Concentration dependence of 1-palmitoyl-lysoPtdCho total influx into the retina and one representative brain area (parietal cortex) during perfusion with HBSS medium. Inset: Eadie-Hofstee plot for the graphical determination of V_{max} and K_m. The values so obtained, together with *PS* data, were used to determine by the equation 4 of Smith et al. [19]. K_d, i.e. the constant describing the non-saturable uptake reported in Table 2.

and lipid extracts were processed and fractionated as previously described [22]. The radioactivity distribution among metabolic products was as follows: aqueous phase, 10.1%; neutral lipids and free fatty acids, 14.8%; lysoPtdCho, 45.4%; PtdCho, 22.1%; other phospholipids, 7.5%.

4. Discussion

The long-term objective of our work is to determine the mechanism by which lipid substances are transported through BBB or BRB. This study presents a quantitative analysis of Pam-lysoPtdCho transport across the BRB and BBB of the rat. Our results demonstrate that Pam-lysoPtdCho is able to cross retinal and cerebral microvasculature, and that the uptake is dependent on the albumin concentration of the perfusion medium. The apparent transport affinity is region-dependent. At the same BSA concentration, Pam-lysoPtdCho showed higher *PS*s than palmitate or docosahexaenoate [17,18]. K_m values were approximately 45-fold greater than those for palmitate in myocytes [23], higher than those for neutral amino acids such as phenylalanine or tryptophan, but similar to those of valine and threonine [19]. One consequence of the high K_m values (range 0.085–0.5 mM), indicative of a lower Pam-lysoPtdCho transport affinity at the BRB and BBB, is that the transport system in the rat is not saturated with lysoPtdCho at normal plasma concentrations (0.2–0.25 mM), except during hypercholesterolemia when lysoPtdCho plasma levels increased [11].

Retinal and cerebrovascular Pam-lysoPtdCho transport, from the plasma albumin complex to inside the endothelial cells and glia–neuron network, can be described quantitatively by a model that in principle includes a saturable and non-saturable diffusion component. The multistep process could be quite similar to that hypothesized for fatty acids in heart myocytes [23]. Substrate transport across the luminal surface membrane implies dissociation from albumin in solution, translocation, partial metabolic utilization across endothelial cells, transport through the abluminal endothelial membrane and extracellular matrix, and finally uptake by the glia-neuron plasma membrane prior to undergoing terminal, intracellular acylation-deacylation reactions. Our data cannot discriminate which step(s) of the long sequence the kinetic constants refer to. However, considering (i) the very small pool size of retinal and cerebral lysoPtdCho (in the rat retina, lysoPtdCho was undetectable [25], and in the rabbit brain and rat cortex it ranged between 0.4 mol % and 3.1 weight % of total phospholipids, respectively [26,27]), (ii) its slight non-saturable component of uptake, and (iii) its fast metabolic transformation, we favor the idea that simple membrane diffusion governs Pam-lysoPtdCho exchange across the endothelial membranes.

The observed saturable component of uptake could be attributed to the sum of intracellular metabolic processes rather than the membrane transport carrier. The major metabolic pathways for the intracellular removal of lysophospholipids have been identified as: (i) reacylation by acyltransferase; (ii) transacylation of two lysophospholipid molecules to form PtdCho, and (iii) hydrolysis by lysophospholipases to form free fatty acids and glycerophosphoryl compounds. A survey of available kinetic data in the literature suggests that in CNS lysophospholipids are rapidly converted into PtdCho (major metabolic route), and partially broken down to glycerophosphoryl products and unesterified choline [11]. The apparent K_m values for brain microsomal acyltransferase and transacylase were determined as 107 μM and 104 μM for 1-acyl-lysoPtdCho, with V_{max} of 6.1 and 1.06 nmol/min/mg, respectively [28,30]. In the rat retina, we reported an initial velocity of 0.67 nmol/min/mg for oleoylCoA:1-acyl-lysoPtdCho acyltransferase [29]. Rod outer segments revealed higher acyltransferase activity than that of lysophospholipase [20]. In addition, lysophospholipase A_1 of rat brain membrane fraction showed a specific activity of 32.6 nmol/30 min/mg [31], and when purified from bovine brain it has a V_{max} of 0.55 μmol/min/mg [32].

Table 2
Kinetic parameters for saturable (V_{max}, K_m) and non-saturable (K_d) component of 1-acyl-L-1-([1-^{14}C]palmitoyl)-2-lysoPtdCho transport into nervous system regions of rats

Regions	V_{max} (nmol/s/g)	K_m (μM)	K_d (ml/s/g × 10^4)
Retina	0.36 ± 0.04	230 ± 65	0.58 ± 0.2
Optic nerve	0.89 ± 0.01	330 ± 98	4.03 ± 1.9
Optic tract	0.97 ± 0.02	305 ± 66	5.50 ± 2.1
Lateral geniculate	0.58 ± 0.01	180 ± 45	0.62 ± 0.2
Superior colliculus	0.32 ± 0.06	95 ± 21	0.91 ± 0.3
Parietal cortex	0.39 ± 0.09	85 ± 19	1.30 ± 0.4
Striatum	0.37 ± 0.07	210 ± 61	0.92 ± 1.0
Hippocampus	0.31 ± 0.06	112 ± 34	2.78 ± 0.9
Olfactory bulb	0.37 ± 0.08	502 ± 108	7.22 ± 2.7

Values (means ± S.E.M., with $n = 3$) were obtained by fitting equations *1* and *4* of Smith et al. [19] to *PS* data and 1-palmitoyl-lysoPtdCho concentrations in the perfusate. The kinetic constants were calculated from uptake values measured after 20 s. In a separate experiment, the uptake values were compared at 11 and 20 s, respectively. *PS* were found to be identical. Hence we assumed that the 20 s uptake values corresponded to *initial* uptake.

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