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COMMENTARY

ROLE OF CONCENTRATIVE LEUKOTRIENE TRANSPORT SYSTEMS IN THE CENTRAL NERVOUS SYSTEM

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The leukotrienes, a family of unsaturated fatty acids, are potent mediators of smooth muscle contractility, vascular tone and permeability, and the functions of leukocytes and other cells [1]. Leukotrienes are synthesized by distinct enzymatic pathways from the 5-hydroperoxyeicosatetraenoic acid (5-HPETE) generated from arachidonic acid by the 5-lipoxygenases of polymorphonuclear leukocytes, macrophages, mast cells, some epithelial cells, and as yet unidentified cells of the central nervous system [1, 2]. The initial C6-peptidoleukotriene biosynthetic product is leukotriene C_4 (LTC_4) from which LTD_4 and LTE_4 are derived by sequential peptidolysis [1, 2]. LTC_4 and, in lesser amounts, LTD_4 and LTE_4 are produced by rat brain slices incubated *in vitro* with ionophore and arachidonic acid [2, 3]. *In vivo* studies have shown that reperfusion of ischemic cerebral tissue results in elevations of the local content of LTC_4 and LTD_4 [4]. The neural cellular sources of LTC_4 and LTD_4 have not been determined, but it has been suggested that a substantial portion of the leukotrienes formed in the latter experiments [4] was derived from leukocytes that had entered the damaged brain tissues [5]. However, there is convincing evidence that leukotrienes exist in normal brain in certain regions, including the median eminence and other parts of the hypothalamus [2, 6]. In contrast to all other cell sources that contain no detectable leukotrienes until they are exposed to stimuli that release arachidonic acid and activate the 5-lipoxygenase, the hypothalamic cells have preformed stores of LTC_4 that have been identified by fluorescent anti-LTC_4 antibodies [2, 6].

Leukotriene C_4 is concentrated by an active transport system within the choroid plexus, which is an organ within the ventricular system of all mammals [7]. The choroid plexus secretes the large majority, if not all, of the cerebrospinal fluid (CSF) and transfers substances between blood and CSF [8]. In contrast, LTC_4 in the extracellular space of brain does not seem to be concentrated by brain cells themselves [7].

In normal brain tissue, leukotriene C_4 may be a neuromodulator that elicits the rapid release of luteinizing hormone (LH) from rat anterior pituitary cells, as contrasted with the much slower release achieved by LH releasing hormone [6]. There is some evidence that both LH and LTC_4 are present in nerve cell bodies in the preoptic area and may possibly be located in the same neurons [6].

The C6-peptidoleukotrienes appear to have two major effects on the vasculature of the central nervous system. First, intraparenchymal injections of as little as 20 ng of LTC_4 into brain cause increased permeability to circulating albumin [9, 10]. Second, LTC_4 and LTD_4 constrict cerebral arterioles in anesthetized mice [11]. As will be discussed below, LTC_4 persists unchanged for hours in the nervous system and is only slowly metabolized to LTD_4 and LTE_4, but not readily oxidized to inactive products, so that prolonged vascular effects would be predicted. Because of the cerebrovascular constrictor and edema-promoting activities of the C6-peptidoleukotrienes [9–11], it has been postulated that they play a role in eliciting cerebral edema after cerebral ischemia and in inducing vasospasms after subarachnoid hemorrhage [7, 12].

When LTC_4 is injected intravenously, it does not appear in the central nervous system in functionally relevant quantities [13, 14]. Both the liver and the kidney rapidly accumulate intravenously injected LTC_4 and excrete it into either the bile or the urine [13–16]. Thus, both the kidney and the liver are involved in removing leukotrienes from the circulation and detoxifying them [13–16].

The purpose of this paper is to discuss physiological and pathological roles of the concentrative leukotriene transport systems of choroid plexus. To do this, we will review briefly the biosynthesis of LTC_4 and its possible functions in the central nervous system, and describe the anatomical bases and functions of the specialized transport systems at the blood–brain and blood–CSF barriers that nourish and protect the brain. The specific role of the LTC_4 concentrative systems of choroid plexus then will be discussed in relation to similar systems in other parts of the body. Finally, we will speculate on the importance of the LTC_4 concentrative systems normally and in selected disease states of the central nervous system.

Blood–brain and blood–cerebrospinal fluid barriers and functions

Teleologically, one would anticipate that the central nervous system, perhaps more than any other organ, must be protected from fluctuations in its

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internal milieu [8]. The complex integrated functions of the central nervous system would seem to dictate that the extracellular space of brain and the CSF, which bathes the inner and outer surfaces of the brain, should not be subject to major fluctuations in composition [8]. It is worth noting that there is no barrier to the diffusion of substances between the extracellular space of brain and the CSF. In fact, the central nervous system has evolved homeostatic and protective functions by using both anatomical and physiological systems [8, 17]. The anatomical systems which isolate the brain from the blood include the cerebral capillaries, which are joined by very tight endothelial junctions, and are termed collectively the blood–brain barrier, and the choroid plexus, of which the epithelial cells also are joined by tight junctions [8, 17]. Moreover, the CSF compartment is surrounded by the arachnoid membrane, which similarly is joined by tight junctions [8, 17]. The brain and CSF thus are isolated from the blood by anatomical barriers. These barriers are effective in retarding the entry by simple diffusion of large (e.g. albumin) and small (e.g. mannitol and salts) water-soluble molecules [8]. In contrast, there is no specific anatomic barrier to the transfer of lipid-soluble substances between blood and brain. These anatomical barriers, if unmodified, would preclude the proper functioning of the brain because of exclusion of water-soluble nutrients required for the maintenance of the viability and functions of neural cells. Specialized systems at the blood–brain and blood–CSF barriers enable the brain to nourish itself adequately [8]. A series of transport systems at both the cerebral capillaries for certain nutrients including glucose, amino acids, and fatty acids, and at the choroid plexus for other nutrients including deoxynucleosides and certain vitamins, selectively transfer these essential substances into the extracellular space of brain and CSF for use by brain cells [8, 17, 18]. The cerebral capillaries and choroid plexuses contain specialized transport systems for nutrients, while the barriers in the same structures tend to "keep out" water-soluble substances that are unnecessary or toxic to neural cells.

The systems described above apparently do not adequately protect the brain from water-soluble substances that exhibit neurotoxicity or alter neural function adversely. For example, penicillin causes seizures when injected directly into the CSF or cerebral ventricles of mammals at relatively low concentrations [19]. These seizures may be intractable and lead to death. Normally, the intact central nervous system does have mechanisms to keep the concentration of penicillin in CSF and the extracellular space of brain at or below approximately 1% of that in the plasma [19]. How does the central nervous system do this? First, as described above, these substances frequently do not penetrate (through) the blood–brain or blood–CSF barrier well [18, 19]. Second, the choroid plexus has an important cleansing role in that it is able to eliminate certain toxic or useless substances from plasma or neural sources by pumping them from the CSF into blood [8, 19]. Choroid plexus epithelial cells resemble renal tubular cells which prevent reabsorption and, in many cases, secrete catabolic waste products or drugs from blood into the urine [8, 19, 20]. However, the directionality of the transfer by choroid plexus is the opposite of that by renal cells. For example, the choroid plexus epithelial cells secrets penicillin from CSF into blood, whereas proximal tubular epithelial cells secrete penicillin from blood into urine [8, 19]. Within the choroid plexus there are many separate active transport systems. There is an active transport system

Table 1. *In vitro* uptake of [^{3}H]LTC_4 by rabbit choroid plexus

Experimental condition	Uptake of [^{3}H]LTC_4 (T/M ratio)	% Control
Control, 3.0 nM	68.4 ± 5.1 (35)	
4°	5.2 ± 0.5 (6)	8*
Dinitrophenol and iodoacetate, both 2 mM	6.0 ± 0.6 (6)	9*
N-Ethylmaleimide, 2 mM	15.2 ± 5.4 (10)	22*
Probenecid, 1 mM	14.5 ± 2.1 (11)	21*
LTC_4, 1.6 μM	55.4 ± 7.3 (7)	81
LTD_4, 1.0 μM	80.6 ± 4.2 (10)	117
Tolazoline, 2 mM	52.5 ± 7.0 (10)	76
Cysteine, 2 mM	65.5 ± 7.1 (10)	96
Glutathione, 2 mM	64.8 ± 5.0 (5)	95
Sodium iodide, 2 mM	65.6 ± 3.1 (5)	96
2.5-min Incubation	10.1 ± 0.8 (5)	15
5.0-min Incubation	19.8 ± 3.4 (10)	29
10.0-min Incubation	43.6 ± 3.7 (26)	64
Forebrain slices	2.2 ± 0.2 (6)	3*
Red blood cells	0.2 ± 0.1 (6)	0*

Choroid plexuses and control tissues and cells were incubated at 37° in medium containing [^{3}H]LTC_4 and other compounds for 15 min, unless otherwise specified [7]. The tissue-to-medium (T/M) ratios are mean ± standard error of the results of the number of experiments indicated in parentheses [7].

* All 15-min percentage values so designated differed significantly ($P < 0.01$) from the control value at 15 min, by Dunnett's test of multiple comparisons with a control.

for transferring iodide and similar smaller inorganic anions from CSF into blood [8]. There are also systems that transfer weak bases, certain amino acids, and certain vitamins, such as riboflavin, from CSF into blood, when concentrations in the CSF exceed safe limits [8, 21, 22]. For the purposes of this review, however, we will focus on the systems that clear weak organic acids from CSF, such as penicillin, salicylate, probenecid, and prostaglandins [19, 23]. Many endogenous substances are also transferred from CSF into blood, including acidic neurotransmitter metabolites [8]. The teleologic explanation for these excretory functions of the choroid plexus is prevention of buildup in the CNS of potentially deleterious substances. The leukotrienes which are vasoactive and cause cerebral edema clearly fit into the category of substances that might cause damage to the central nervous system. Rapid transfer of these substances from CSF into blood would allow their rapid removal and detoxification by either the liver or kidneys.

In general, the transport systems in the choroid plexus which transfer substances from CSF into blood concentrate the substance in the intracellular space by energy-dependent saturable mechanisms [8, 19, 24]. Then, the transported substance leaves the choroid plexus by diffusion or facilitated diffusion and enters the circulation.

Leukotriene transport in the central nervous system

To study the transfer of leukotrienes from the central nervous system into blood, we performed a series of *in vitro* and *in vivo* studies in rabbits [7, 25]. *In vitro,* isolated choroid plexuses obtained from brains of New Zealand white rabbits, were placed individually in 3 ml of artificial CSF containing 5 mM glucose and 3 nM purified synthetic [^{3}H]LTC_4 [7]. In some cases, substances known to alter other transport systems of the choroid plexus were added to the medium. The incubations were carried out in a metabolic shaker at 37° under 95% O_2:5% CO_2 for various times up to 15 min. At the end of the incubation, tissue-to-medium (T/M) ratios were determined, and the nature of the [^{3}H] within the choroid plexus was established. The choroid plexus was able to concentrate [^{3}H]LTC_4 by a saturable energy-dependent system that did not depend on binding or metabolism within the choroid plexus (Table 1). Moreover, this system was specific in that probenecid, but not tolazoline, cysteine, glutathione, or sodium iodide, inhibited the accumulation of [^{3}H]LTC_4 by the system. The uptake of [^{3}H]LTC_4 was not due to red blood cells in the choroid plexus. Thus, the isolated choroid plexus concentrates [^{3}H]LTC_4 by a specific energy-dependent system *in vitro*.

To study the clearance of [^{3}H]LTC_4 from the central nervous system, 2.5 μCi of [^{3}H]leukotriene (36 Ci/mmole) was injected along with [^{14}C]mannitol (as a marker of passive diffusion) and, in some cases, 0.9 mg probenecid or other substances into the left lateral ventricle of sodium-pentothal anesthetized rabbits [25]. After 2 hr, the ratio of [^{3}H] to [^{14}C]mannitol and the nature of the [^{3}H] was determined in CSF and brain. After the intraventricular injection of [^{3}H]LTC_4, [^{3}H] was removed rapidly from the cerebrospinal fluid and the central nervous system by a probenecid-sensitive system (Table 2). These results are all the more striking in view of the fact that the molecular weight of LTC_4 is approximately three times the molecular weight of mannitol (M_r 180). Although there was slight oxidation of [^{3}H]LTC_4 and the formation of small amounts of LTD_4 and LTE_4 in both CSF and brain, these metabolites amounted to less than 10% of [^{3}H] recovered; the majority of the radioactivity that remained in the central nervous system after 2 hr was in fact [^{3}H]LTC_4 [25].

Thus, after the intraventricular injection of [^{3}H]LTC_4, [^{3}H]LTC_4 was transported from the central nervous system by a probenecid-sensitive mechanism much more rapidly than [^{14}C]mannitol. Second, this was apparently not due to metabolism of the [^{3}H]LTC_4. Third, this system was specific in that it was not inhibited by the weak base tolazoline, which blocks the transport of weak bases from CSF.

In summary, the choroid plexus is a small organ in the central nervous system that behaves in many

Table 2. Ratio of [^{3}H]/[^{14}C] in tissue divided by ratio of [^{3}H]/[^{14}C] in injectate (modified from Ref. 25)

	Control (5)	Probenecid (4)	Cysteine (4)	Unlabeled LTC_4 (Carrier) (4)
CSF	0.31 ± 0.01	0.67 ± 0.02*	0.25 ± 0.02	0.40 ± 0.2*†
Choroid plexus	1.5 ± 0.2	1.3 ± 0.1	0.5 ± 0.1*	3.0 ± 0.06
Left brain	0.35 ± 0.04	0.50 ± 0.03*	0.16 ± 0.01*	0.36 ± 0.02
Right brain	0.34 ± 0.07	0.48 ± 0.03	0.17 ± 0.01	0.32"± 0.02
Total‡	0.33 ± 0.02	0.60 ± 0.02*	0.20 ± 0.01*	0.39 ± 0.02

Rabbits were injected intraventricularly with 2.5 μCi [^{3}H]LTC_4, 0.3 μCi [^{14}C]mannitol and, in some cases, 0.9 mg probenecid, 1.8 mg cysteine, or 1.4 μg unlabeled LTC_4 [25]. After 2 hr, the [^{3}H] and [^{14}C] content of the tissue was determined, and the ratio of [^{3}H]/[^{14}C] in the tissue was divided by the ratio of [^{3}H]/[^{14}C] in the injectate. Each value is the mean ± S.E.M. of the results of studies of the number of rabbits shown in parentheses. Ratios which are less than 1.0 signify that [^{3}H]LTC_4 was cleared more rapidly than the [^{14}C]mannitol marker of passive diffusion in the central nervous system.

* $P < 0.05$ (two-tailed Dunnett's test).

† The concentration of unlabeled LTC_4 in the CSF withdrawn after 2 hr was 0.14 μM.

‡ Total equals total [^{14}C] and [^{3}H] radioactivity in CSF, choroid plexus and brain.

respects like a kidney, with a great capacity for bidirectional transport of diverse molecular species. The choroid plexus transports several important nutrients for the functioning of the brain, such as vitamins, by stereospecific systems [7]. In addition, there are much less specific systems in the choroid plexus, and possibly at the blood–brain barrier, that transport weak organic acids and other substances from CSF into blood [8, 19, 23]. The transport system for [^{3}H]LTC_4 in choroid plexus resembles the transport system for penicillin G, prostaglandins, and other weak organic acids in the choroid plexus [8, 19, 23]. At the end of 2 hr in comparable experiments, [^{14}C]penicillin achieved ratios of 0.06 relative to mannitol, compared to a ratio of 0.31 for [^{3}H]LTC_4 [25, 26]. This suggests that penicillin G, which is a molecule approximately one-half the size of LTC_4, may have a higher affinity for the carriers of the weak organic acid system in choroid plexus and thus is more efficiently transferred out of CSF. We were unable to measure saturation of LTC_4 transfer from CSF with unlabeled LTC_4 because of limited availability of LTC_4. (We did note though that intraventricular injections of several micrograms of LTC_4 did not kill or apparently grossly impair the ability of the rabbit to function, at least over 2 hr.) Two hours after the unlabeled LTC_4 was injected, the concentration in the CSF was 0.14 μM (Table 2). In conclusion, LTC_4 is one of many substances that not only does not penetrate the blood–brain barrier well but, if it does or is made locally, is exported from CSF and brain. This would presumably be advantageous to the animal since C6-peptido-leukotrienes, which may have important physiological functions in the hypothalamus [2, 6], might be injurious when formed in excessive amounts. Moreover, maintaining a very low concentration of the leukotrienes in the CSF and hence the central nervous system might preserve the capacity of neuroendocrine receptors to sense small changes in the local concentration of LTC_4.

Speculation

Subarachnoid hemorrhage, in which blood enters the cerebrospinal fluid, frequently is complicated by severe intractable arterial vasospasm that may lead to a stroke syndrome and death [12, 27]. This complication often occurs several days after the initial hemorrhage. There has been substantial speculation that the spasm may be due to leukotrienes or other vasoactive mediators in the CSF generated by normal tissue or leukocytes stimulated by elements of the blood in the CSF [7, 12, 27]. If the choroid plexus is involved in transferring the leukotrienes from CSF into blood, why would LTC_4 or other mediators accumulate? One possibility is that blood and substances in blood, such as fibrinogen, may interfere with choroid plexus function. Many other possibilities exist and require further study.

A second possible consequence of LTC_4 in the CSF is that LTC_4 itself may cause vasospasm in the vessels of the choroid plexus. This could interfere with the ability of choroid plexus to transport LTC_4 as well as other substances, because of effects on blood flow to the choroid plexus. Thus, elevated concentrations of LTC_4 in CSF for prolonged periods may interfere with its own removal and detoxification, as well as the bidirectional transfer of other substances, including nutrients.

Third, it is possible that the aging choroid plexus which tends to become fibrotic and appears increasingly dysfunctional with age [28] may be unable to transfer LTC_4 and other substances between CSF and blood. A consequence of this might be higher resting levels of LTC_4 and resultant interference with the capacity of LTC_4 to act as a local neuroendocrine substance or neuromodulator. Further studies along these lines are required to answer these questions definitively.

Finally, the biochemistry of the LTC_4 system in choroid plexus needs further work. It is very difficult to understand the broad specificity of the organic acid transport and secretion systems in choroid plexus or, for that matter, the kidney. Is there one organic acid system with incredibly broad specificity—i.e. the ability to transfer molecules as diverse as LTC_4, penicillin, riboflavin, prostaglandins and probenecid; or are there multiple systems? How do these systems work on a molecular level? Are there congenital or acquired disorders of these systems?

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