four cardionatrins are natriuretic, it is expected that they contain most of the sequence of cardionatrin I. Compositional analysis of cardionatrins III and IV (Table 1) indicated that these proteins extend up to and include tyrosine at position 150.

Identification of the start points for cardionatrins I and III in procardionatrin confirmed that these products are derived from a common precursor, cardionatrin IV. They are part of a growing list of cleavage products that include a 106-residue peptide (*16*), a 73-residue peptide (*17*), and numerous versions of the carboxyl terminal portion that we have isolated as cardionatrin I. It is not clear which of these are intermediates in a maturation pathway in vivo and which, if any, are artifacts of preparation.

The very small peptides (atriopeptins) isolated and sequenced by Currie *et al.* (*3*) were not present when rat atria were subjected to acid extraction by the procedures we used and by those of de Bold and Flynn (*6*), and it is possible that peptides shorter than cardionatrin (residues 1 to 28) are artifacts of extraction (*18*). Our extraction procedure is similar to that used for the extraction of pituitary hormones by Bennett *et al.* (*8*), who showed that the structural integrity of peptides in rat anterior pituitary was maintained during acid extraction and purification. It is possible, therefore, that cardionatrins I to IV are the natural cleavage products from procardionatrin.

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Leukotriene C_4 Transport by the Choroid Plexus in Vitro

Abstract. *Nanomolar concentrations of peptidoleukotrienes evoke sustained cerebral edema and arterial constriction. Peptidoleukotrienes are thus considered to play an important role in eliciting cerebral edema after cerebral ischemia and vasospasm after subarachnoid hemorrhage. It was hypothesized that the choroid plexus, the locus of the blood–cerebrospinal fluid barrier, might minimize the vasoactivity of locally generated or systemically derived leukotrienes by transporting leukotrienes from cerebrospinal fluid into the blood. Consistent with this hypothesis, leukotriene C_4 in vitro was transported into and released from isolated rabbit choroid plexus by a system that was specific, energy-dependent, probenecid-sensitive, and depressed by cold temperatures. The accumulation of leukotriene C_4 in the choroid plexus was not dependent on tissue binding or metabolism of leukotriene C_4.*

The leukotrienes include the pharmacologically active peptidolipids, one of which, the slow reacting substance of anaphylaxis, is now known to be a mixture of leukotrienes C_4, D_4, and E_4 (*1*). Leukotrienes are synthesized via hydroperoxyeicosatetraenoic acid (HPETE) generated from arachidonic acid by the 5-lipoxygenase pathway (*1*). When leukotriene C is injected intravenously, it does not appear in the central nervous system in functionally relevant quantities (*2*). Whether this is due to poor entry of this large, water-soluble molecule or to active transport of leukotrienes out of the central nervous system, or both, is not clear. However, leukotriene C (LTC_4) is synthesized in gerbil brain after cerebral ischemia and reperfusion (*3*). Moreover, products of the lipoxygenase pathway such as 5-HPETE are detectable in cerebrospinal fluid (CSF) of patients with subarachnoid hemorrhage (*4*). Because of the extremely potent cerebral vasoconstrictor (*5*) and edema-promoting activities (*1*) of the leukotrienes, they have been considered to play an important role in eliciting cerebral edema after cerebral ischemia (*3*) and vasospasm after subarachnoid hemorrhage (*4*).

To eliminate these potent substances, the central nervous system might either metabolize them to inactive products or transfer them to the blood for subsequent metabolism in liver and kidney. We hypothesized that a mechanism for transferring leukotrienes out of the central nervous system might be contained in the CSF compartment within the choroid plexus, a locus of the blood-CSF barrier (*6*). The choroid plexus is involved in the transfer of many water-soluble substances between blood and CSF by separate, specific, carrier-mediated mechanisms (*6, 7*). Our study of the isolated rabbit choroid plexus shows that (i) there is a specific energy-dependent system for uptake of LTC_4 in the choroid plexus, and (ii) this uptake does not depend on binding or intracellular metabolism of the LTC_4, within choroid plexus. Furthermore, the uptake system has a relatively low affinity but high transport capacity for LTC_4. These results are compatible with the choroid plexus serving as a locus of transport of LTC_4 from CSF into blood in vivo.

[14,15-^{3}H]Leukotriene C_4 ([^{3}H]LTC_4; 39 Ci/mmol) (New England Nuclear) was purified by high-performance liquid chromatography (HPLC) before being used (*8*). The choroid plexuses (~5 mg each), obtained from brains of New Zealand White rabbits (1.5 to 2.0 kg) killed with intravenously injected pentobarbital, were individually placed in 3 ml of artificial CSF (in plastic flasks) containing 5 m*M* glucose and 3.0 n*M* [^{3}H]LTC_4 (*9, 10*). In some cases, various other substances were added to the medium. The incubations were carried out in a metabolic shaker at 37°C under 95 percent O_2 and 5 percent CO_2 for various times up to 15 minutes. At the end of the incubation, each choroid plexus was

Table 1. Uptake of [^{3}H]LTC_4 by rabbit choroid plexus in vitro. Choroid plexuses (and other tissues) were incubated for 15 minutes at 37°C unless otherwise indicated. Uptake is expressed as the ratio of radioactivity in the tissue to that in the medium, and the values are given as means ± standard error for *n* experiments.

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

*Values differ significantly from the control value ($P < 0.01$) by Dunnett's test of multiple comparisons with a control.

wiped on a glass slide, weighed, and homogenized in 0.5 ml of water. The radioactivity in the tissue homogenates and media was determined, and the ratio of radioactivity in the tissue to that in the medium was calculated (*10*). The effect of substances added to the medium on the uptake of [^{3}H]LTC_4 and the time course of the uptake by the isolated choroid plexuses are indicated in Table 1. As control tissues, rabbit forebrain slices (0.25 mm) and rabbit red blood cells were incubated for 15 minutes in medium containing 3.0 n*M* [^{3}H]LTC_4 under the same conditions (*9, 10*). The uptake of [^{3}H]LTC_4 by forebrain slices and red cells was 3 percent or less than that of the isolated rabbit choroid plexus (Table 1) (*11*).

The possibility that structural alterations in the [^{3}H]LTC_4 had occurred in the tissue or medium during the incubations was tested as follows: The choroid plexuses were incubated for 15 minutes in 3.0 n*M* [^{3}H]LTC_4. After the incubation, three choroid plexuses from each rabbit were homogenized in 1.0 ml of a chilled (4°C) solution containing equal volumes of water and isopropanol. Before and after the incubations, the incubation medium was diluted with an equal volume of isopropanol. The radioactive material was extracted from the sample and subjected to HPLC (*8*). Direct injection of [^{3}H]LTC_4 from one experiment into the HPLC system indicated that a mean of 73 percent ($n = 3$) was genuine [^{3}H]LTC_4. When choroid plexus extracts and media were injected into the HPLC system, recoveries ranged from 80 to 110 percent of the total radioactivity. In the tissues, 54 ± 2 percent (mean ± standard error) ($n = 5$) of the radioactive label eluted with genuine LTC_4. In the media incubated alone and those in which choroid plexuses were incubated for 15 minutes, 55 ($n = 2$) and 51 percent ($n = 2$), respectively, of the radioactive label eluted with LTC_4. In another experiment, direct injection of [^{3}H]LTC_4 into the HPLC system yielded a mean of 86 percent as genuine [^{3}H]LTC_4. In this experiment, when choroid plexus extracts were injected, 66 percent ($n = 2$) of the radioactivity was associated with LTC_4. In the media in which choroid plexuses were not incubated and those in which they were incubated, 78 ± 2 percent ($n = 3$) and 71 percent ($n = 2$), respectively, of the radioactivity was associated with LTC_4. In these experiments, approximately 50 percent of the radioactivity not associated with LTC_4 was associated with LTC_4 sulfone, and the remainder was associated predominantly with an 11-*trans* isomer of LTC_4, as determined by HPLC. Recovery of the 11-*trans* isomer and LTC_4 sulfone is not unusual after such manipulations. Together, these experiments show that there is only minimal, if any, metabolism of [^{3}H]LTC within the choroid plexus. It was not possible to determine the half-saturation concentration for LTC_4 uptake because of limited availability of unlabeled LTC_4. However, concentrations of 1.6 μ*M* LTC_4 and 1.0 μ*M* LTD_4 did not significantly decrease [^{3}H]LTC_4 accumulation (Table 1).

The possibility that uptake of [^{3}H]LTC_4 by choroid plexus was due to extensive tissue binding was investigated by two techniques (*12*). First, the choroid plexuses were incubated for 15 minutes in 3 n*M* [^{3}H]LTC_4. The tissue was then homogenized in 1.5 ml of artificial CSF. After centrifugation at 50,000*g* for 15 minutes at 4°C, 89 ± 6 percent ($n = 5$) of the ^{3}H in the homogenate was present in the supernatant. Second, the choroid plexuses, after incubation for 10 minutes in 3.0 n*M* [^{3}H]LTC_4, were rinsed in artificial CSF (2 seconds) and then transferred for a second incubation at 37°C (or 4°C) into artificial CSF (release medium). After the second incubation, the ratio of ^{3}H in the medium to the total ^{3}H in the tissue and the medium was determined. At 5 and 10 minutes, 21 ± 1 percent ($n = 5$) and 39 ± 5 percent ($n = 8$), respectively, of the ^{3}H had been released from the choroid plexus. When the second (release) medium was kept at 4°C, only 10 ± 3 percent ($n = 6$) of the ^{3}H was released at 10 minutes. Furthermore, addition of metabolic poisons, such as dinitrophenol and iodoacetate, or performing the incubations at 4°C greatly decreased the accumulation of [^{3}H]LTC_4 (Table 1). Thus, a significant portion of the ^{3}H was not irreversibly bound inside or on the surface of the choroid plexuses.

Concentrative transport systems for leukotrienes in liver and kidney have been described (*13*). These systems accumulate leukotrienes for excretion or for inactivation by metabolism, or both. The transport system in choroid plexus probably has functions similar to those in liver and kidney. We postulate that the system in choroid plexus is an active transport system and transfers leukotrienes from CSF into blood. There is substantial indirect evidence in favor of this hypothesis. First, many compounds that are transported from CSF to blood via choroid plexus in vivo are concentrated in the isolated choroid plexus in vitro (*6, 7*). Second, the accumulation of [^{3}H]LTC_4 in the choroid plexus is not due to tissue binding or metabolism of the [^{3}H]LTC_4. Third, the uptake system in choroid plexus is specific in that iodide and the weak base tolazoline, both of which are transported on separate specific carrier-mediated transport systems (*6*), do not affect the uptake of LTC_4. However, probenecid, which blocks the weak acid transport systems in the choroid plexus, had a significant effect (Table 1) (*6*). The system for transporting leukotrienes in choroid plexus is similar to the system for transporting penicillin from CSF into blood in that both systems are probenecid-sensitive

and have relatively high half-saturation concentrations for inhibition (K_i for penicillin ~ 50 μM; K_i for LTC_4 >> 1 μM (Table 1) (*6, 10*). Certain prostaglandins are also transported from CSF into blood by a probenecid-sensitive system in choroid plexus (*6*). The relation of the leukotriene transport system in choroid plexus (Table 1) to the transport systems for penicillin, prostaglandins, and other weak acids remains to be determined. Fourth, the fact that leukotrienes, when injected into the blood, achieve extremely low levels in brain and presumably CSF (*2*), is probably due to their poor penetration into CSF and active transport back into blood. Penicillin is handled in this fashion by the choroid plexus in vivo (*6, 10*).

The transport system for leukotrienes in choroid plexus can probably transport leukotrienes effectively out of the extracellular space of brain, since there is no anatomical barrier to the diffusion of substances from the extracellular space of brain into CSF (*7*). It is also possible that cerebral capillaries have a comparable system for direct transfer of leukotrienes from extracellular space of brain into blood.

Finally, we speculate that the transport system for leukotrienes in choroid plexus would protect the central nervous system from the vasopermeability-inducing and vasoconstrictor activities of leukotrienes synthesized in brain after cerebral ischemia (*3*) or when blood enters the CSF as in a subarachnoid hemorrhage (*4*). Blood in CSF may also interfere with choroid plexus function. Answering these questions will require in vivo studies to define the function and specificity of this system and the effect of disease states on leukotriene transport.

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Mechanism of Aeration in Rice

Abstract. *Mass flow of air to the submerged parts of the plant constitutes the major mechanism of aeration in partially submerged rice. It is proposed that the flow of air results from reduction of pressure in the air-conducting system of the plant caused by consumption of oxygen and solubilization of respiratory carbon dioxide in the surrounding water.*

Most of the world's rice is grown on land that is flooded for at least part of the season. These conditions greatly limit the availability of O_2 to the submerged parts of the plant. Aeration problems are especially severe for deep-water or floating rices, which grow in water as deep as 6 m and reach a height of up to 7 m (*1*). It has been postulated that the aeration requirements of the submerged organs of rice and other plants tolerant of partial flooding are met by O_2 entering the above-water parts of the leaves and moving by diffusion through internal air spaces to the submerged organs (*2, 3*). In rice these air spaces are particularly well developed in the culms (*4*) and roots (*5*). Using excised leaves, we have demonstrated that continuous air layers trapped between the hydrophobic surface of rice leaves and the surrounding water form a low-resistance pathway for gas movement (*6*). We now report that air is moved to the submerged parts of the plant through the external air layers and internal air spaces primarily by mass flow rather than diffusion and provide evidence that this mass flow is driven by solubilization of respiratory CO_2 in water.

To demonstrate mass flow of air to the submerged parts of a rice plant, leaf tips protruding from the water were placed in a small leaf chamber that was inverted into a submergence tank and connected to a volumeter. Movement of air into the plant was monitored by recording the disappearance of air from the headspace of the leaf chamber with an angular transducer (Fig. 1). The rate of air intake was 4.9 ml/hour during the first period of light (Fig. 2). When the lights were turned off after 3 hours the rate of air intake abruptly increased fourfold but gradually declined over a period of 3

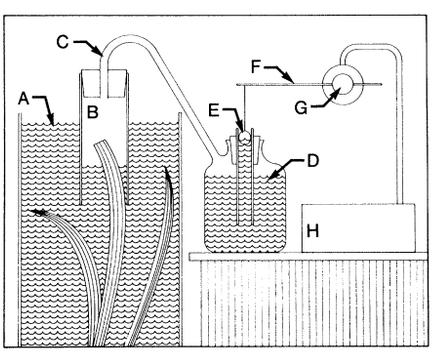

Fig. 1. Experimental setup to measure mass flow of gases. A rice plant was placed in a submergence tank (A) filled with water. One to six leaves were introduced into a leaf chamber (B) with an initial headspace volume of 50 ml. Before the start of the experiment the tips of these leaves were cut at the same height so that only a 5- to 6-cm portion of each blade was above the water level in the leaf chamber. All other parts of the plant were kept submerged. The leaf chamber was connected with Tygon tubing (C) to a volumeter (D) such that the height of the water column in the glass tube with the Styrofoam float (E) was always equal to the difference between the water level of the submergence tank and that of the leaf chamber. When air was withdrawn from the headspace by the plant the water level in the leaf chamber rose and the height of the water column under the float dropped. The float was linked by a rigid steel rod (F) to the lever of an angular transducer (G) to amplify the movement of the float. The transducer was connected, via a power source and an amplifier, to a chart recorder (H). Displacement of the recorder pen was directly proportional to the changes in the headspace volume of the leaf chamber and was calibrated in milliliters of gas withdrawn from the headspace or expelled into it. Calibration was performed by injecting into or withdrawing from the headspace known volumes of air with a gas-tight syringe inserted into the leaf chamber through the rubber stopper. Every 1 or 2 hours the leaf chamber was lifted from the submergence tank for 1 minute to replace the gases in the headspace with air and to readjust the height of the water column in the volumeter. We verified that the rate of gas movement into the rice plant was not affected by the small increase in pressure in the leaf chamber. The results were essentially the same with atmospheric or a slightly negative pressure inside the leaf chamber.