Choroid Plexus in the Central Nervous System: Biology and Physiopathology

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Abstract. Choroid plexuses (CPs) are localized in the ventricular system of the brain and form one of the interfaces between the blood and the central nervous system (CNS). They are composed of a tight epithelium responsible for cerebrospinal fluid secretion, which encloses a loose connective core containing permeable capillaries and cells of the lymphoid lineage. In accordance with its peculiar localization between 2 circulating fluid compartments, the CP epithelium is involved in numerous exchange processes that either supply the brain with nutrients and hormones, or clear deleterious compounds and metabolites from the brain. Choroid plexuses also participate in neurohumoral brain modulation and neuroimmune interactions, thereby contributing greatly in maintaining brain homeostasis. Besides these physiological functions, the implication of choroid plexuses in pathological processes is increasingly documented. In this review, we focus on some of the novel aspects of CP functions in relation to brain development, transfer of neuro-humoral information, brain/immune system interactions, brain aging, and cerebral pharmaco-toxicology.

Key Words: Alzheimer; Blood-brain barrier; Brain development; CSF; Inflammatory response; Multidrug resistance; Organic anions.

INTRODUCTION

The choroid plexuses (CPs), located within the brain ventricles, can be viewed as a tight epithelium enclosing a vascularized stroma, and as such form an interface between the blood and the cerebrospinal fluid (CSF). Known as the main site of secretion of this intracerebral fluid, the CPs also have an important capacity of exchange that is accounted for by the specific structural and biochemical properties of the choroidal epithelial cells. CSF secretion is obviously a key feature of the CPs given the different functions of this fluid, which range from buoyancy and intracranial volume adjustment, to buffering of brain extracellular fluid ions and other solutes, to drainage. This secretion is the result of complex inorganic ion exchanges across the tight epithelium of the CP, mainly driven by the activities of the Na\(^+\) K\(^+\) ATPase located at the CSF-facing membrane of the epithelial cells, and of the carbonic anhydrase. CSF production is controlled by a precise neurohumoral regulation of the choroidal blood flow (1). Other functions of the CPs that also contribute to brain homeostasis include the supply of various micronutrients (such as vitamin C, folate, vitamin B12, deoxyribonucleosides) via specific transport mechanisms, the synthesis and secretion in CSF of numerous peptides and proteins, the clearance from CSF of neurotransmitter catabolites, and other harmful compounds (reviewed in 2). Neuroscientists with very different interests have recently reported implications of CPs in a much broader range of physiological and pathological processes. In this review, we will first give a brief updated description of CP anatomy and histology and then focus on some of their novel or better understood functions, namely, in relation to brain development, transfer of neuro-humoral information, brain/immune system interactions, brain aging, and cerebral pharmaco-toxicology.

ANATOMY AND HISTOLOGY OF THE CHOROID PLEXUS

In vertebrates, CPs are papillary structures protruding in the ventricular CSF. They occur in each of the 4 major cisternae developing from the median wall of each lateral ventricle and from the roof of the third and fourth ventricle. Macroscopically, the 3 types of CPs dissected from an adult rat brain are easily distinguishable. The lateral ventricle CP is a thin undulating veil, whereas the fourth ventricle CP is highly lobulated and more complex (Fig. 1). The third ventricle CP, much smaller in size, has an intermediate appearance. All 3 possess a similar extensive vasculature. Microscopically, all plexuses share a similar structure consisting of an external simple cuboidal epithelium surrounding a vascular bed embedded in a loose connective tissue (Fig. 2). The former, continuous with the ependyma lining the adjacent ventricular walls, differs fundamentally from it by the presence of apical continuous tight junctions, and provides, therefore, the anatomic basis for the restrictive blood-CSF barrier. The epithelial layer folds into villi around each of the numerous capillaries, forming fronds of various shapes that project into the CSF and thus greatly increase the ventricular surface area of the plexus. Furthermore, both apical and basolateral membrane surfaces are enhanced, respectively, by the tightly packed border of microvilli and the elaborate infoldings and interdigitations found at the basal portion of the intercellular spaces. Considering these ultrastructural elements in a morphometric analysis of the lateral ventricle CP, Keep and Jones (3) estimated...
Fig. 1. Stereomicrographs of choroid plexuses isolated from adult rat brain. The lateral ventricle choroid plexus (top panel) appears as a thin undulating veil, with a narrow linguina (upper portion) and a thicker glomus containing the larger vessels (bottom right portion). The fourth ventricle choroid plexus (bottom panel) is more bulky and lobulated, and presents 2 “arms” which, in situ, extend in the lateral recesses of the fourth ventricle. Both display an intense vascular bed. The enlargement (inset) highlights the extensive capillary network. Note the epithelial lining (about 10-μm-thick), which can be seen on the outer surface, overlying a capillary. Scale bar: 1 mm.

The total apical surface of all combined CPs to be 75 cm², and the basolateral CP to be about 25 cm² in a 30-day-old rat. Such large areas of exchange point out a more important role of the CPs in brain homeostasis and blood-brain exchanges than previously thought. Strengthening this view is the 5- to 13-fold amplification in the brush border membrane surface of the epithelial cells that has been described in 5 primate species (4). The polarized phenotype of the choroidal epithelium, concomitant with a high number of mitochondria (4), are common features of secreting/reabsorbing epithelia. This large mitochondrial volume is assumed to provide the metabolic capacity required for maintaining ionic gradients and for CSF secretion, and may be linked in particular to the energy-driven activity of the apical Na⁺ K⁺ ATPase.

The fibrovascular core separated from the epithelium by a continuous basal lamina and connected with the external leptomeningeal membranes contains the blood
Fig. 2. Schematic cross-section of 2 choroid plexus villi illustrating the main morphological and histological features of the choroidal tissue. The choroid plexus consists of numerous fronds projecting into the CSF, each frond composed of several villous processes. The outer simple cuboidal epithelium lays on a basal lamina and delimits an inner stromal core of connective and highly vascularized tissue. It derives from the adjacent ependyma lining the ventricle walls and differs from it by the presence of apical tight junctions. The epithelial cells are polarized and the apical membrane facing the CSF bears uneven borders of irregular microvilli and consistent groups of cilia. The lateral membranes of these cells display complex infoldings at their basal ends. Each villus contains a large capillary of the fenestrated type with very thin endothelial walls. The stromal connective tissue is composed of a loose network of collagen fibers, secreted by occasional elongated fibroblasts. Globular macrophages, rich in phagolysosomes, are also present in the stromal core and are distinct from the star-shaped dendritic cells. A few of the latter “squeeze” between the basal lamina and the choroid epithelium and extend processes between epithelial cells. Polymorphic eiplexus cells, or Kolmer cells, lie on the ventricular surface of the epithelial cells, in close association with the microvilli border.

vessels embedded in a loose network of collagen fibers, which are produced by the occasional fibroblasts present in this stroma. In contrast to the tight parenchymal capillaries that form the anatomic basis of the blood-brain barrier (BBB), the CP capillaries supplying each villus are unusually large and have been classified as fenestrated capillaries. The thin endothelium displays regions of extreme attenuation and membrane apposition, also called closed fenestrae. Tracer studies using horseradish peroxidase, or microperoxidase, have demonstrated the absence of a barrier between the blood and the extracellular space of the stroma (5).

In the connective core, the presence of other cell types, such as cells of the monocyte/macrophage lineage, has been established for some time. More recent immunophenotypic studies on rat CPs have characterized these cells and described a dense network of dendritic cells expressing major histocompatibility complex (MHC) class II molecules, as well as a smaller number of resident tissue macrophages reactive for ED1 and ED2 antigens.
Star-shaped dendritic cells were also reported in human CP where a few of them localized between the basal lamina and the epithelium, extending some of their processes between adjacent epithelial cells (8, 9). Furthermore, lymphocytes T but not B were identified in the stromal connective tissue of CP isolated from the brain of AIDS patients and also from nonpathological brain (10).

The intraventricular epiplexus cells or Kolmer cells, closely associated with the choroidal epithelium, are regarded as part of this tissue. These free motile cells display diverse morphological shapes ranging from round, to polar, to stellate, and a variable number of pseudopodal processes. They rest on the apical microvilli of the epithelial cells and show ultrastructural features typical of active macrophages, such as vacuoles and lysosomes (reviewed in 11).

ONTOGENY OF THE CHOROID PLEXUS AND CHOROIDAL INFLUENCE ON BRAIN DEVELOPMENT AND MATURATION

Choroid Plexus Embryology and Morphogenesis

The 2 components of the CPs, i.e. the external epithelium and the vascularized connective core, have different embryological origins. The neuroepithelium lining the neural tube evaginates to give rise to the choroidal epithelium, while the stromal core originates from the adjacent primitive mesenchyme. The embryology of the CP, as described for human, rat, mouse, or chicken, classically divides in several phases (see 2 for a review). The CP first represents a simple fold of pseudostratified tall columnar epithelium, overlying a loose mesenchyme filled with angioblasts forming ill-defined capillaries. When the plexus expands and becomes lobulated, the epithelium changes to simple low columnar cells containing abundant glycogen and the stromal blood vessel grow in a more organized fashion. As CP villi develop further, the epithelial cells turn to cuboidal cells decorated with apical microvilli and the glycogen content decreases. The stromal mesenchyme fills up with connective tissue fibers.

Morphogenesis of the CP is an early event in CNS development and occurs in vertebrates before the pontine flexure appears on the 5 vesicle-segmented neural tube. Times of earliest histological evidence of choroidal differentiation, as reviewed for several species by Catala (12), indicate that the metencephalic plexus (fourth ventricle) generally appears first, followed by the telencephalic plexuses (lateral ventricles), and finally the diencephalic plexus (third ventricle).

Choroidal determination is yet a much earlier event. Using heterotypic transplantation in the quail–chick chimeric system, Wilting and Christ (13) showed that 1) the prospective telencephalic epithelial cells from quail embryos, when implanted in contact to the mesenchyme of the coelomic cavity of chicken embryos, formed a normal CP; 2) these cells were determined at least 3 days before this CP starts to evaginate; and 3) in contrast, prospective choroidal stroma failed to induce the formation of CP from other regions of the neural epithelium. Using a cell culture system, Thomas and Dziadek (14) were able to identify within the homogenous undifferentiated neural epithelium of mouse embryos, distinct cell populations that had the potential to differentiate into choroidal epithelial cells. Segmentation of the neuroepithelium into regions restricted to CP cell lineage occurred between day 8.5 and day 9.5 of gestation, i.e. 4 days before CPs differentiate.

The sequence of cues responsible for the choroidal determination of neuroepithelium is still unknown. Wnt genes, known as patterning signals in the vertebrate brain, may be involved in the morphogenesis of the telencephalic CPs. The absence of development of these plexuses observed in the extra-toes1 mouse mutant, which carries a deletion in the transcriptional regulator Gli3, is associated with a down regulation of the Wnt genes in the cortical hem, a boundary region between the hippocampus and the plexus (15). Other genes, whose expression is spatially correlated with the presumptive CP regions, are likely to play a role in the morphogenesis of these structures. High molecular tropomyosins, which are actin regulatory proteins, may be involved in the dynamic regulation of cell shape or motility during choroidal evagination into the ventricles (16). Bone morphogenetic proteins, by inducing both a local reduction in the cell proliferation rate and an increase in apoptosis, could generate the thin layer of columnar epithelial cells from the pseudostatified neuroectoderm (17).

Differentiation and Maintenance of the Choroidal Phenotype

In CP as in most tissues, the importance of mesenchyme–epithelial interactions in the differentiation of epithelial cells during embryonic development has been established. A model of choroidal differentiation has been proposed that is based on the temporal and spatial pattern of expression of the insulin-like growth factor II (IGF-II) and transthyretin (TTR), a thyroid hormone transporter, which in the brain is synthesized exclusively by the plexus epithelial cells (18). IGF-II is produced by the prospective choroidal mesenchyme before CP develops in the brain vesicles. It would act as a differentiation factor on the adjacent primordial epithelial cells and induce in these cells the early synthesis of TTR. As CP organogenesis proceeds, IGF-II production shifts progressively from the mesenchyme to the epithelial layer. A high density of IGF-II receptor binding sites has been reported in the fetus rat CP (19), and mitosis of choroidal epithelial
cells in culture is stimulated by IGF-II that they secrete (20). Together, these data indicate that IGF-II acts first as a paracrine factor to induce CP phenotype, and later as an autocrine factor that regulates cell division and possibly maintains the phenotype. IGF-II gene is a target for HFH-4, a transcription factor, highly expressed in the mouse presumptive CP and during CP epithelial differentiation (21). Whether HFH-4 is directly involved in IGF-II transcription, and thus participates in the signaling of CP differentiation, remains to be established.

In addition to the paracrine mesenchymal inductive effect, the basement membrane composition is also a crucial factor for epithelial differentiation. In vitro studies on cultured fetal choroidal epithelial cells have demonstrated the importance of the basement membrane components, and growth factors such as TGFβ2, on the cell growth rate and polarized phenotype (22).

Finally, CP differentiation should be regarded as a cross-talk process between the mesenchyme and the epithelium, rather than just a one-way induction mechanism. In the above mentioned study of quail-chicken chimera, Wilting and Christ (13) reported that the graft mesenchymal blood vessels originating from the chicken coelomic cavity displayed a typical choroidal phenotype induced by the overlying epithelium. Vascular endothelial growth factor (VEGF), which is produced by the choroidal epithelium at high level, may be involved in the induction and maintenance of these fenestrations by interacting with the endothelial cells expressing VEGF receptors (23).

Paracrine Influence of Choroid Plexus on the Developing Brain

An intriguing question is why CPs develop so early during embryogenesis, unless they are required for the normal brain development. This view is certainly not a recent one since Luschka in 1855 referred to the CP as “placenta cerebralis,” basing this statement on the huge amounts of glycogen that are present in this structure during the early stage of its development.

More likely, CPs provide the maturing brain with a controlled extracellular environment. Interestingly, in the immature brain, the blood-CSF barrier already displays structural and functional features of the mature CP (see 24 for review). Functional tight junctions between epithelial cells are present as soon as the plexus begins to differentiate (25). Some mechanisms are even specific to the fetal CP, such as an intracellular tubulocisternal endoplasmic reticulum route for albumin, which may account for the high protein concentration in fetal CSF compared with the adult (24). Choroidal epithelial carbonic anhydrase and Na⁺ K⁺ ATPase, both involved in CSF secretion, have been shown to be expressed respectively in the human and rat embryo at a very early stage (26, 27). High levels of CHIP28 water channel (aquaporin 1) mRNA are also present in the CP at similar early times of development (28), suggesting that CSF secretion is already functional. Although the postnatal increase in Na⁺ K⁺ ATPase activity and in CSF secretion rate reported in rat and sheep by several authors (29, 30) suggested that at least in some animal species this function is rather immature, the CSF turnover rate measured in sheep was in fact 3-fold higher in the fetus than in the adult, as a result of the concomitant increase in CSF volume (30).

The CP may also serve as the crucial source of morphogens, mitogens, and trophic factors into the developing brain. This view is supported by the localized high expression of IGF-II in the embryonic CP. Expression of type 1 IGF receptors is rather ubiquitous in the developing brain, and especially high in the floor plate of the hindbrain, in close proximity to the fourth ventricle CP. Bondy et al thus hypothesized that IGF-II secreted by the plexus diffuses to the receptors on the floor plate cells and activates their function of guidance in spinal axon growth (31). Recently, more direct evidence for the role of CP as a morphogen secreting tissue was obtained in the developing forebrain. Radial migration of cerebral cortical neurons from the ventricular zone or subventricular zone to the cortical plate is governed by gradients of soluble diffusing signals, such as secreted semaphorins, netrins, or Slit proteins, which provide attractive or repulsive guidance cues to growing axons. Such a factor, Slit2, is expressed and presumably secreted during prenatal development by the telencephalic CP and the septum (32). Hu (33) demonstrated in vitro that a soluble factor, secreted by the fetal CP and immunologically related to Slit2, is likely to diffuse through the CSF and aid in establishing a gradient of a repulsive cue guiding cortical neurons away from the ventricular surface.

The role of CP in neurite outgrowth was also documented in the hindbrain at the level of the cerebellum. This structure is closely apposed to the fourth ventricle CP and its complex development and maturation occurs in 2 phases, before and after birth, when the plexus has already largely expanded in the cistern. The cerebellum is known to be highly vulnerable to retinoid teratogenesis, and both retinoid deficiency and excess lead to developmental defects (34). Although its capacity for retinoic acid synthesis is very low, the cerebellar tissue expresses the different receptors for this lipid mediator (35). Therefore, the precise modulation of retinoid supply seems of crucial importance for normal cerebellum development. Using explant cocultures of cerebellum and fourth ventricle CP from fetal and infant rat, Yamamoto et al (35) demonstrated the secretion by the plexus of a soluble neurite-growth promoting activity that could be mimicked by pure retinoic acid. Furthermore, the activity of the retinoic acid-synthesizing enzyme in the CP was
high and biphasic, with 2 maxima coincident with the major events of cerebellar morphogenesis.

As for retinoids, deficit or excess of thyroid hormones during the critical periods of cerebellum development leads to abnormalities in this tissue (36). Although direct evidence has not yet been provided, one could speculate that the above-mentioned TTR expression in the embryonic choroidal epithelial cells might provide the developing cerebellum with the required thyroid hormone supply. Furthermore, the transcriptional activity of thyroid hormone receptors is known to be modulated by heterodimer formation with retinoic acid receptors and a cooperative effect between thyroid hormone and retinoic acid on neuronal differentiation has been demonstrated (37). In the absence of a temporally regulated secretion, signaling via the thyroid hormone during critical periods of cerebellar development could be achieved via the receptor heterodimerization process.

**IMPLICATION OF CHOROID PLEXUS IN NEUROHUMORAL MODULATION OF THE BRAIN**

CSF may participate in volume transmission, a mode of information transfer complementary to the classical wire (pre- to post-synaptic) transmission. In volume transmission, neurotransmitters (or in an extended view of the definition, neuromodulators), act on extrasynaptic receptors on cells that are distant from the site of release. The increasing recognition of the CSF circulatory system as a pathway of information and signaling transfer (38) is supported by 1) the presence of CSF contacting supraependymal serotoninergic/GABA-ergic terminals, a neuronal network within the CSF on the floor of the third ventricle, and specific receptors for neuroactive compounds in the leptomeninges; 2) the significant levels of various neuroactive substances in the CSF; 3) the easy access from CSF to neurons located in the periventricular gray matter across the nontight ependyma; 4) the peculiar location of the circumventricular organs, which implies a role for CSF in their neuroendocrine regulatory functions; and 5) the rapid and direct access of ventricular CSF-borne compounds to pial, cisternal and velae membranes, and arterial walls (38, 39).

**Participation of Choroid Plexus in the Control of Intracerebral Neurotransmitter Concentration**

By means of specific transport mechanisms, CP regulates the CSF concentration of some neurotransmitter precursors, such as choline (40), which is the limiting substrate in the acetylcholine synthesis, or glutamine (41), which is both a product of glutamate detoxification by astrocytes and a precursor for glutamate and GABA synthesis in glutamatergic and GABA-ergic neurons, respectively. The involvement of CP in signal termination of GABA and neuropeptides has also been proposed. Thus, isolated CP accumulates GABA, and the CP as well as the other CSF-brain interfaces largely express GAT2, one of the transporters involved in GABA uptake (42, 43). Another aspect of the involvement of CP in the control of neurotransmitter action is the prevention of interference between peripheral and CSF endogenous neuroactive amines via the monoamine oxidase, L-amino-acid decarboxylase, and catechol-O-methyltransferase activities, which form an enzymatic barrier located at the CP epithelium (2). It is noteworthy that the CP epithelium possesses specific receptors for neuromediators, and particularly the metabolotropic serotonin receptors 5-HT2C that uniquely localize on CP (44), suggesting a modulation of CP functions by centrally released neurotransmitters.

**Role of Choroid Plexus as a Source of Humoral Factors in the Brain**

CP location is ideal for the CP epithelium to be a source of active signaling molecules circulating in CSF, either by direct (endogenous) synthesis, or by controlled transfer from the blood. This function is illustrated by the following examples.

A well-characterized molecule involved in signaling, and secreted in CSF by the CP, is the major thyroid hormone carrier protein, TTR. During evolution, TTR first appeared in the CP. Much later, the liver also became a source of TTR. In human as in other mammals, the liver and the CP remain the only sites of synthesis for plasma TTR and brain TTR, respectively (45). Thyroxine (T4), which is the precursor of the active triiodothyronine, is a lipophilic hormone that circulates in plasma essentially bound to TTR, albumin, and thyroxine binding globulin. However, these complexes are not transported into the brain, and T4 entry involves continuous choroidal TTR synthesis and secretion in CSF. The binding of T4 to CSF TTR and the diffusion of the complex into the brain parenchyma result in a control of the pool of T4 in the cerebral extracellular space, and in an efficient prevention of nonspecific thyroxine partitioning into lipid-rich cerebral membranes (45, 46, and related papers from this group). The efficiency of choroidal TTR in transferring T4 from the blood to the brain, and in keeping normal cerebral steady-state levels of T4, has been confirmed in vivo using a TTR competitive inhibition method (46) and transthyretin-null mice (47). These mutant mice lacking TTR have a 50% lower plasma concentration of total T4 compared with wild type mice. However, the mutant animal was able to adapt and displayed near normal levels of T4 in all tissues investigated by the authors, with the striking exception of the brain, as a consequence of the lack of TTR in the CPs. Thyroid hormones are not only critical for embryonic brain development, as previously discussed, but are also important in preventing mental retardation in children and are required throughout adult life. A reduction in the CSF concentration of TTR has
be associated with depression (48) and lead poisoning, which results in a loss of cognitive abilities in children (49). A strong lead accumulation by choroidal epithelial cells in culture induced a decrease in both choroidal synthesis and secretion of TTR, as well as in T4 transport, which suggests that a defect in TTR production at the CP may result in severe pathologies via a dysregulation of brain thyroid hormone homeostasis (49).

A second example of CP-mediated signaling is illustrated by beta-trace protein, also called prostaglandin D (PGD) synthase. This protein, involved in the cerebral metabolism of eicosanoids, synthesizes prostaglandin D2 (PGD2), which is a major mediator of sleep. In the brain of human and other mammals, the major sites of PGD synthase production are the epithelium of the CP and some cells in the meninges (50). PGD synthase is secreted in the CSF as an enzymatically active protein. PGD2 receptors involved in sleep regulation are localized in the subarachnoid space at the ventroorostral surface of the basal forebrain, i.e., downstream from the ventricular CSF in which both PGD synthase and PGD2 circulate (50). PGD2 is also associated with functions other than sleep, such as modulation of body temperature. Other binding sites for PGD2 are also found in leptomeninges outside the sleep-sensitive area and on the CP itself (51). Thus, by regulating the production of PGD2, PGD synthase appears to be a key enzyme in the control of physiological sleep and other neuroendocrine functions related to this prostanoid. In addition to its enzymatic activity, PGD synthase is also a member of the lipocalin superfamily, and as such may promote the circulation of lipophilic signaling molecules (other than PGD2) in the CSF and extracellular space. This other potential function of choroidal PGD synthase remains to be closely investigated.

In both adult rat and human brain, the expression of IGF-II is mainly restricted to CP and meninges (18). Insulin-like growth factor binding protein 2 (IGFBP-2), the main binding protein for IGF-II in the brain, is coexpressed with IGF-II in the adult central nervous system. IGFBP-2 is readily detected in CSF, mainly bound to IGFBP-2, and can be delivered to various distant cerebral structures in which IGF-II receptors are expressed on both neurons and glial cells (19, 52). In particular, IGF-II function may involve the maintenance of nondividing cells by promoting the formation of neural processes and the maintenance of the glia limitans integrity. It also induces the restoration of tissue homeostasis following brain injury. Indeed, an increased secretion of IGF-II in CSF was described in the acute phase of brain injury, followed by a transport of the trophic factor, bound to IGFBP-2, to the injured area. In a later phase, when a reactional local synthesis of IGF-II occurred on the site of injury, the CSF level of IGF-II decreased (52).

The last example illustrates the role of CPs in the central action of leptin. This circulating hormone is synthesized by adipose tissue, gastric epithelium, and placenta, and among several functions controls appetite and body weight. Its mechanism of action involves its interaction with a receptor located in the arcuate nucleus of the hypothalamus, which leads to a decrease in the hypothalamic level and activity of neuropeptide Y, a major orexigenic molecule (53). Besides the arcuate nucleus and other hypothalamic nuclei, a high level of leptin receptors or leptin binding sites in CP from many species, including human, has been consistently observed by a large number of laboratories. Whereas the main hypothalamic receptor has a long intracytoplasmic domain involved in the transduction of the signal generated by leptin binding, the receptor located at the CP has a short intracytoplasmic domain (54). Interestingly, when labeled leptin was given intravenously, only the CP, the arcuate nucleus protected by a complete blood-brain barrier, and the nearby median eminence (a circumventricular organ lacking a blood-brain barrier) were strongly labeled (55). Because a hypothetical leak from the median eminence to the surrounding hypothalamic area has not been documented, it is tempting to speculate that the short form of CP receptor acts as a blood-to-CSF transporter for leptin, allowing its delivery to the arcuate nucleus and other periventricular areas along the ventral part of the third ventricle. In support of this hypothesis, direct evidence that leptin can indeed be transported from blood to CSF, in an intact form and by a saturable process, has been provided at the level of the lateral CP using the isolated perfused sheep CP technique (56). With regard to the physiopathology of obesity, although it remains speculative, a defect in this transport would explain the lack of weight regulation in obese individuals, in which the abundance and the sequence of hypothalamic leptin receptor appear normal. As reviewed by Tartaglia (54), studies of leptin concentration in CSF and plasma of lean and obese patients show evidence for a blood-to-CSF transport of leptin occurring via a saturable mechanism, and for a much lower CSF-to-serum ratio of leptin in obese than in lean individuals. Leptin plays a role not only in the central regulation of food intake, but also of other major functions such as reproduction. It is further involved in the regulation of inflammatory immune responses mediated by macrophages (57). The presence of these cells in CSF-containing compartments highlights the potential importance of CSF-borne leptin in CNS immune regulation. In all accounts, in view of the different regulatory pathways in which leptin is involved, the puzzling strong expression of leptin receptor at the CP deserves additional investigation to fully elucidate its exact function.

These examples emphasize the importance of CP in signaling mechanisms in the brain. They are not exhaustive and other putative signaling compounds include...
growth hormone and prolactin, possibly transported from blood to brain at the PC, or adrenomedullin and arginine vasopressin, known to be synthesized in the choroidal epithelium.

CHOROID PLEXUS AS A COMPONENT OF THE NEUROIMMUNE SYSTEM

The concept of an immune privileged status of the brain has evolved considerably over the last years when the relationship between the cerebral extracellular fluid, the CSF, and the lymphatic system were documented, together with leukocyte trafficking into the brain and the participation of neural cells in immune reactions.

Given their peculiar localization at the interface between the blood and the CSF/brain compartment, and the abundance and diversity of cells of the lymphoid lineage present in these structures, CPs are thought to be key players for mediating interactions and/or signaling between the peripheral immune system and the brain. This has been conspicuously highlighted by the restricted activation pattern elicited in rat by systemic subseptic doses of LPS (lipopolysaccharide endotoxin), which resulted in a rapid induction of the interleukin IL-1β and TNF-α (tumor necrosis factor) only at the CP, leptomeninges, and circumventricular organs devoid of tight capillaries (58).

Choroid plexuses and leptomeninges have a moderate expression of the LPS binding protein CD14, which is strongly and rapidly induced upon injection of a low septic dose of LPS (59). Other studies from the same laboratory reported in these immune challenge conditions an upregulation of TNF-α, of both the constitutive p55 form and to a lesser degree the p75 form of TNF-α receptors, and of IL-6, another proinflammatory cytokine. This rapid and transient activation pattern, observed in the CPs and also in CVOs, leptomeninges, and around blood vessels, was the first step of a biphasic process, spreading throughout the brain in a second phase. A similar biphasic cytokine induction pattern with a much slower time course was also observed in a natural disease model i.e. the rat infected with the parasite Trypanosoma brucei (60). This sequence suggests that through a coordinated local induction of proinflammatory cytokines, CP has a role in the transfer of information between the peripheral immune system and the brain.

Another role of the CPs in cell-mediated immune responses is implied by the wide expression of MHC molecules. Choroidal epithelial cells constitutively express MHC Class II molecules (8, 9), and class I molecules were induced in mice choroidal epithelium upon infection with rabies virus (61). Furthermore, these epithelial cells are indeed capable in vitro of presenting foreign antigen and stimulating T lymphocyte proliferation through a MHC Class II restricted mechanism (62). In addition, accessory molecules important for leucocyte adhesion, such as the cell adhesion molecules ICAM-1 and VCAM-1, are present on the epithelial cells, at low level in healthy CPs where they localize on the apical membrane, and are upregulated in animal models of experimental autoimmune encephalomyelitis (63). The ligand for L-selectin, another leucocyte adhesion molecule, has also been described on the choroidal epithelium (64). Immunophenotyping of intrastromal and intraepithelial dendritic cells in human CP indicated that these cells also express MHC Class II molecules, but the lack of B7 co-stimulatory factors suggests that they are maintained in a quiescent state, possibly via an autocrine secretion of IL-10 (8). Finally, epiplexus or Kolmer cells, besides a function as scavenger cells based on their phagocytic activity, also display both classes of MHC antigens. These antigens are induced under endotoxin challenge and the number of epiplexus cells increases concomitantly (reviewed in 11).

The antigen presentation capacity displayed by different cell components of the choroidal tissue thus suggests that CPs possess an intrinsic surveillance system based on resting and activated cells, which may function as a defense against blood-borne pathogens, but also against antigens arising in the CSF. Upon endo/phagocytosis and antigen presentation, the subsequent activation of lymphocytes could occur locally or peripherally after migration of the dendritic cells. Because CPs form an interface between 2 circulating fluid compartments, they are highly exposed to various immune stimuli from both sides. Hence, activation processes at the CP are extremely complex and may be balanced by inhibitory chemokines or cytokines, such as IL-10.

The fact that an inflammatory reaction triggered by pathogens is prominent in the CP is not surprising given the tropism of various bacteria, parasites, and viruses for these structures (65). Thus, for some of these pathogens, CPs may offer a privileged access to the brain. Recent studies sustain this hypothesis. Neisseria meningitidis is a pathogen responsible for septicemia and meningitis. In the course of CNS invasion, this bacteria has been shown to accumulate preferentially into the CP, to bind to the endothelial cells of the choroidal capillaries, and to a lesser extent to attach to the endothelium of the meningeal vessels. This suggests a possible direct access from blood to the CSF across the CP (66). In rats infected with Trypanosoma brucei, the parasite does not significantly enter the brain but localizes in the CP where it triggers an important synthesis of various cytokines able to generate damage in the CP and also on the ventricular walls after diffusion into the CSF. Interestingly, only the lateral ventricle CPs were affected by the parasite, suggesting a difference in tropism for the 3 types of CPs (60). Viruses also target the CPs, as previously reviewed for Sendai virus, the Lymphocytic Choriomeningitis virus (65), and mumps virus, a member of the paramyxovirus group. Both human immunodeficiency virus (HIV-1) and human
T cell leukemia virus-1 (HTLV-1) are retroviruses associated with neurological disorders. For HIV-1 these disorders range from mild cognitive impairment to the severe AIDS dementia complex. HTLV-1 induces a chronic myelopathy (TSP/HAM) with demyelination and axonal loss in the pyramidal tract and medulla. HIV-infected T lymphocytes and monocytes are often found in the stroma, as well as in supraepithelial regions of the CP (9, 10), suggesting that CPs may be a preferential pathway of entry for infected cells into the brain. Accordingly, in the early stage of infection, HIV-1-positive cells are described in the subarachnoid and perivascular spaces (67). In later stages, when neurological symptoms appear, free infected cells are also present in the CSF of HIV-1 and HTLV-1 infected patients (68, 69).

Finally, the hypothesis that CP may also be involved in the entry of activated, myelin-directed autoreactive T lymphocytes in the brain during the course of multiple sclerosis (MS) deserves some attention. Activated T lymphocyte infiltration in the brain results in the formation of demyelination plaques that are responsible for the clinical symptoms. Because these plaques are frequently located in the periventricular area, the CP may constitute a preferential way for T lymphocytes to reach these structures. This view is supported by the presence of T lymphocytes and T lymphocyte chemoattractants in the CSF from patients suffering from MS (70).

How infected leukocytes, or T lymphocytes activated in response to an inflammatory challenge, may cross the epithelium of the CP remains to be ascertained. The strong cytokine upregulation in the different types of cells, including the epithelial cells, may play a role in the destabilization of the barrier function sustained by the tight epithelium. Other possible factors include an induction of nitric oxide synthase activity or the generation of free radicals. The mechanism resulting in the cell passage across the choroidal epithelium is, however, likely to be different from the ICAM-1 and VCAM-1-mediated mechanism of lymphocyte and monocyte transmigration across the parenchymal capillaries because of the strict apical, i.e., CSF facing localization of these adhesion molecules on choroidal epithelial cells. Only a slight induction of VCAM-1 at the basolateral membrane is observed in experimental inflammation model (63). The migration through the choroidal epithelium may depend on other receptors, such as L-selectin ligands, which are present in the CP (64) and on CSF-borne chemokina, as sustained by experiments showing that intracerebroventricular injection of C10 chemokine leads to a recruitment of T lymphocytes at the CP (71). Matrix metalloproteases (MMP) secreted by T lymphocytes are thought to be involved in the breakdown and remodeling of the extracellular matrix and junctional rearrangement necessary for the invasion and transmigration processes. Because CP epithelial cells also synthesize and secrete several forms of MMPs and the MMP inhibitor TIMP-3 (72), a modification of the epithelial MMP/TIMP balance triggered by cytokines may result in an altered epithelial barrier permeability.

**CHOROID PLEXUS AND ALZHEIMER DISEASE**

The relationship between CP functions and Alzheimer disease (AD) deserves some attention. Several lines of evidence indicate that CP is both a target and an actor in the occurrence of the lesions and amyloid deposits seen in the parenchyma and around the blood vessels in AD. Amyloid beta peptide (Aβ), the main component of the amyloid deposits, is present as a soluble form in the CSF and extracellular fluid in physiological condition, albeit at very low level. Evidence for a rapid clearance of the CSF peptide has been obtained in vivo (73). Although a slow passive diffusion of soluble Aβ into blood could occur at the periventricular capillaries, the BBB does not appear to actively participate in CSF-borne peptide removal (74), thus leaving the CP as a likely site of Aβ clearance. CSF inhibits β-amyloid fibril, and CSF proteins are likely to be involved in this effect. Transthyretin has been shown to sequester Aβ and inhibit amyloid aggregation (75). Apolipoprotein J also binds Aβ and may be involved in the removal of this peptide via the low density lipoprotein receptor-related protein-2 (LRP-2), which is expressed by CP epithelium and ependyma (76).

Like any tissue, the CP-CSF system ages and its different functions deteriorate. In AD, the alteration of CSF dynamics and the morphological alterations of the CP are exacerbated. TTR concentration in CSF increases with age, but is significantly lower in AD, raising the possibility of an imperfect physiological sequestration of Aβ in the CSF/extracellular fluid (77). The occurrence of thread- and tangle-like elements in the plexus and ependyma is linked with cortical AD-type lesion (78). Thus CP alterations in AD, in conjunction with the resulting dysfunction of CSF circulation, impaired clearance of deleterious substances, and decreased supply of neurotrophic and neuroprotective elements, such as growth factors and vitamins, may favor the development of the neuropathological features responsible for AD.

**PHARMACO-TOXICOLOGICAL ASPECT OF CHOROID PLEXUS FUNCTIONS**

Because the cells that form both the BBB and the blood-CSF barrier are sealed by tight junctions, the cerebral bioavailability of a compound will mainly depend on its ability to dissolve and diffuse through the cell membrane, that is, its lipid solubility and size, unless it is substrate for a specific transport mechanism. Other factors include the degree of binding to, and the rate of dissociation from circulating proteins or red blood cells, and the recognition by efflux proteins, such as the product of the multidrug resistance (mdr) gene, P-glycoprotein.
at the level of the BBB. For drugs that can clear the epithelial barrier, CPs allow a direct access from blood to the CSF. However, CPs are also involved in the neuroprotection of the brain in that they have the capacity to excrete various xeno- and endobiotic organic compounds into the blood and to metabolize drugs and toxins.

**Role of CPs in Drug Delivery into the Brain**

The major site for exchange between the blood and the brain parenchyma is the BBB, yet CPs play a more significant role in brain drug delivery than previously thought for several reasons. As previously mentioned, the choroidal surface available for exchange between the blood and the CSF is largely increased by the basolateral infoldings, and the apical microvilli of the choroidal cells. In addition, CPs appear more permeable to highly polar compounds than BBB, thus allowing a limited paracellular diffusion to occur. Highly hydrophilic drugs, such as stavudine (D4T), can slowly but significantly diffuse into the CSF, whereas they are excluded at the BBB (79). Choroid plexuses also constitute a direct access to the ventricles, ependyma and subependymal tissue, leptomeninges, velae, outer layers of pial vessels, and perivascular spaces (39, 73). Targeting of perivascular and meningeal areas by drugs entering the brain through the CP is of importance in the treatment of infectious diseases, such as meningitis or AIDS. As mentioned before, the major productive cells in the brain that should be reached by antiretroviral agents are macrophagic cells primarily found in the subarachnoid spaces and periventricular spaces, especially during the early phase of the infection (67). Simple diffusion from CSF into brain extracellular fluid can occur in most ventricular areas. A large parenchymal diffusion may be achieved for micronutrients and proteins that are continuously secreted from CPs in the same manner as transthyretin, but will be restricted to a variable extent for drugs or xenobiotics delivered into the brain by the CSF route, depending on factors such as variable CSF concentration, BBB efflux, cellular uptake or binding, and metabolism (80).

**Influence of Choroid Plexus in the Cerebral Bioavailability of Drugs and Toxins**

The active export and metabolic processes located at the CPs can influence not only the rate of entry into the CSF, but also the overall cerebral biodisposition. The efflux for both organic anions and cations at the CP started to be documented in the sixties. Since then the list of substrates efficiently removed at the CP has expanded and includes exogenous compounds, such as benzylpenicillin, quinolones, methotrexate, anionic pesticides, contrast agents, cimetidine, but also endogenous neurotransmitter metabolites such as 5-hydroxyindolacetic acid and homovanillic acid. The mechanisms involved have been characterized mainly by uptake studies on isolated CPs. The determination of the kinetics of uptake, energy, and sodium dependency substrate specificity pointed out that different transport proteins are involved in these processes (reviewed in 81). However, the molecular identity of the transporters and their exact subcellular localization in the choroidal cells have started unraveling only recently. A member of the organic anion transport protein (oatp) family is the first demonstrated in the CP. This oatp family comprises several proteins that mediate an inwardly directed flux of organic anions at the cell membrane. Their amino acid sequences and plasma membrane distribution have been characterized mainly in hepatocytes and in renal epithelial cells, where they contribute to the excretion of various endogenous and exogenous compounds into the bile and urine. Proteins of the oatp family have been sought in CP and oatp1 has been identified at the apical membrane of the rat CP epithelial cells, which is its only cerebral localization (82). This transport protein accepts a broad range of amphipathic substrates and acts as an energy-dependent, sodium-independent antiporter with HCO₃⁻ or glutathione. Another member of the oatp family, oatp-2, with a strong affinity for digoxin, has been cloned from rat brain, and has been shown to be present at the luminal and abluminal membranes of the endothelial cells forming the BBB, and at the basolateral side of the CP epithelium (83). In view of its localization, oatp-2 seems to be involved in brain influx rather than efflux processes. As T4 is recognized by oatp-2, one function of this transporter at the CP could be to regulate the entry of T4 into the epithelial cells for its further secretion in the CSF as a complex with locally produced TTR, as previously discussed. Whether oatp-2 can interfere with the CSF-to-blood efflux of organic anions mediated by the apical oatp-1 remains to be investigated. Finally, another transport protein has been located at the apical membrane of the choroidal epithelial cell in rat, and is closely related to ROAT1/OAT1 (renal organic anion transporter 1). This transporter does not belong to the oatp family and mediates the cellular uptake of smaller anionic compounds such as 2,4-dichlorophenoxyacetic acid, a typical substrate, salicylate, and p-aminohippurate. In the CP, as in the kidney, this OAT transports organic anions in exchange for dicarboxylate (alpha-ketoglutarate), a process coupled with a Na⁺/dicarboxylate cotransport that maintains the intracellular pool of glutarate (84). The presence in CP of other members of this family, such as hOAT1, a human isoform analog to the rat renal OAT1, and the murine brain specific OAT-3, has been postulated and remains to be ascertained.

Among cations exported from CSF at the CP, cimetidine is peculiar in that organic anions, but not cations, compete for its transport, indicating that one of the choroidal organic anion transport systems is involved in its efflux (81). However, at least one specific cation transport
system exists at the apical membrane of CP epithelial cells, as demonstrated by the specific concentrative uptake of a model organic cation, tetraethylammonium, at the apical membrane of cultured choroidal epithelial cells (85). The molecular identity of the transport mechanism at the apical membrane is not yet elucidated.

The CP is also a major site for drug metabolism in the brain (reviewed in 80). This feature, together with specific pathways of metabolite disposition present at the CP epithelium, may modulate the cerebral biodisposition of drugs. Drug metabolism is a mainly hepatic multi-phase process catalyzed by several multigenic families of isoenzymes, and in most cases reduces the toxic or pharmacological activity of xenobiotics, increases their hydrophilicity, and results in their elimination from the body by the biliary tract and the kidney. Phase I or functionalization phase includes 1) hydroxylation and de-alkylation reactions catalyzed by enzymes such as cytochrome P-450s, flavin mixed-function oxidase, and in some cases monoamine oxidases, 2) epoxide hydrolysis, or 3) reductive metabolism. Phase II, or conjugation phase, leads to the formation of a polar metabolite by addition of a sugar (glucuronic acid), a sulfate moiety, or a tripeptide (glutathione), onto the functional group of the parent compound or of a primary metabolite. Phase III or export phase allows the cellular extrusion of the formed conjugates. During the oxidation or reduction steps, reactive species, such as carcinogenic epoxides and free radicals, are generated. Hence, epoxide hydrolases and antioxidant enzymes, such as superoxide dismutase and glutathione peroxidase, also play a significant detoxification role in controlling the deleterious metabolites produced by drug metabolism, as well as by cellular endogenous metabolic pathways. The drug metabolizing enzymes investigated to date in CPs are mainly located in the choroidal epithelium, with the exception of some cytochrome P-450 isoenzymes found in the endothelium. In rat, the specific activities of several conjugation enzymes and of the membrane-bound epoxide hydrolase do reach hepatic levels (80, 86). The large GSH concentration and antioxidant enzyme activities contribute to maintain low steady-state levels of oxidant species in the CPs, emphasizing the importance of glutathione metabolism at the blood-CSF barrier. Recently, the pharmaco-toxicological relevance of this drug metabolism capacity was investigated. The use of an in vitro model of the choroidal epithelium suitable for transport studies demonstrated that the choroidal epithelium could act as a complete metabolic blood-CSF barrier toward some xenobiotics. Also, relevant to phase III of drug metabolism, a polarized blood-facing efflux of conjugates occurs at the choroidal epithelium, and involves a specific transporter with characteristics similar to those of the multidrug resistance associated protein (MRP) family members (86). In agreement with these functional data, MRP1 has been shown to be largely expressed at the CP epithelium, and to localize at the basolateral membrane (87, 88). That other members of the MRP family participate in conjugating the efflux at the CP cannot be excluded.

The most documented transport and metabolic mechanisms influencing the cerebral bioavailability of drugs at the choroidal epithelium are summarized in Figure 3. One report mentioned the presence of P-glycoprotein in epithelial cells of the CP with a subapical localization, oatp2, present on the basolateral membrane of the epithelial cells. At least 1 organic cation transporter located on the apical membrane is responsible for the CSF to blood efflux of organic cations (C). A coupled metabolism/efflux process at the choroid plexus results in the intraepithelial conjugation of lipophilic xenobiotics (X), followed by the polarized elimination of the polar metabolites (GS-X) at the blood-facing membrane via MRP, thus providing another mechanism of brain protection. MRP may also be responsible for the cellular efflux of some of the compounds taken up at the apical membrane by the organic anion transporters.

![Fig. 3. Summary of the different metabolic and transport processes at the blood-cerebrospinal fluid barrier influencing the cerebral biodisposition of drugs. A large range of organic anions (A) are efficiently cleared from the CSF into the blood by various transporters localized on the ventricular surface of the choroidal epithelium. These proteins belong to at least 2 different families, oatp and OAT, and have little overlapping substrate specificity. An organic anion influx from the blood into the epithelial cell can be mediated by another oatp member, oatp2, present on the basolateral membrane of the epithelial cells. At least 1 organic cation transporter located on the apical membrane is responsible for the CSF to blood efflux of organic cations (C). A coupled metabolism/efflux process at the choroid plexus results in the intraepithelial conjugation of lipophilic xenobiotics (X), followed by the polarized elimination of the polar metabolites (GS-X) at the blood-facing membrane via MRP, thus providing another mechanism of brain protection. MRP may also be responsible for the cellular efflux of some of the compounds taken up at the apical membrane by the organic anion transporters.](http://jnen.oxfordjournals.org/)

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**Summary of the different metabolic and transport processes at the blood-cerebrospinal fluid barrier influencing the cerebral biodisposition of drugs.** A large range of organic anions (A) are efficiently cleared from the CSF into the blood by various transporters localized on the ventricular surface of the choroidal epithelium. These proteins belong to at least 2 different families, oatp and OAT, and have little overlapping substrate specificity. An organic anion influx from the blood into the epithelial cell can be mediated by another oatp member, oatp2, present on the basolateral membrane of the epithelial cells. At least 1 organic cation transporter located on the apical membrane is responsible for the CSF to blood efflux of organic cations (C). A coupled metabolism/efflux process at the choroid plexus results in the intraepithelial conjugation of lipophilic xenobiotics (X), followed by the polarized elimination of the polar metabolites (GS-X) at the blood-facing membrane via MRP, thus providing another mechanism of brain protection. MRP may also be responsible for the cellular efflux of some of the compounds taken up at the apical membrane by the organic anion transporters.
removed from the CSF at the CP. The apical uptake of these compounds appears to be mediated by oatp-1, whereas the basolateral export is likely to be MRP dependent (86, 88).

CONCLUSION

The CNS requires a carefully controlled internal environment for its normal functions from early development to the adult and senescent stages. As illustrated by the few examples reviewed, the CPs greatly contribute to this crucial homeostasis via different mechanisms. In addition to being the major source of CSF, they form a structural barrier, a finely regulated selective interface for the delivery of nutrients, hormones, and trophic factors as well as for the transduction of peripheral signals, an enzymatic protective barrier and a clearance site for deleterious compounds and catabolites, and an immunologically active interface. From these aspects, CPs fulfill hepatic-like, renal-like, endocrine and immune functions within the brain. It is thus not surprising that the human pathologies identified to date (in which CPs are known or suspected to be involved), range from hormonal dysregulation, to toxicologic insults, to inflammatory and infectious diseases, to neurodegenerative processes. The exact delineation of CP involvement in these diseases calls for additional research and may lead to the identification of new targets for more efficient treatments. Similarly, as the molecular mechanisms of transport, metabolism, and efflux processes occurring at the CP become better understood, they should become the basis for the design of therapeutic strategies in order to improve the bioavailability of several classes of pharmacologically active compounds in the CNS.

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