The Choroid Plexus-Cerebrospinal Fluid System: From Development to Aging

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The function of the cerebrospinal fluid (CSF) and the tissue that secretes it, the choroid plexus (CP), has traditionally been thought of as both providing physical protection to the brain through buoyancy and facilitating the removal of brain metabolites through the bulk drainage of CSF. More recent studies suggest, however, that the CP-CSF system plays a much more active role in the development, homeostasis, and repair of the central nervous system (CNS). The highly specialized choroidal tissue synthesizes trophic and angiogenic factors, chemorepellents, and carrier proteins, and is strategically positioned within the ventricular cavities to supply the CNS.
with these biologically active substances. Through polarized transport systems and receptor-mediated transcytosis across the choroidal epithelium, the CP, a part of the blood-CSF barrier (BCSFB), controls the entry of nutrients, such as amino acids and nucleosides, and peptide hormones, such as leptin and prolactin, from the periphery into the brain. The CP also plays an important role in the clearance of toxins and drugs.

During CNS development, CP-derived growth factors, such as members of the transforming growth factor-β superfamily and retinoic acid, play an important role in controlling the patterning of neuronal differentiation in various brain regions. In the adult CNS, the CP appears to be critically involved in neuronal repair processes and the restoration of the brain microenvironment after traumatic and ischemic brain injury. Furthermore, recent studies suggest that the CP acts as a nursery for neuronal and astrocytic progenitor cells. The advancement of our knowledge of the neuroprotective capabilities of the CP may therefore facilitate the development of novel therapies for ischemic stroke and traumatic brain injury. In the later stages of life, the CP-CSF axis shows a decline in all aspects of its function, including CSF secretion and protein synthesis, which may in themselves increase the risk for development of late-life diseases, such as normal pressure hydrocephalus and Alzheimer’s disease. The understanding of the mechanisms that underlie the dysfunction of the CP-CSF system in the elderly may help discover the treatments needed to reverse the negative effects of aging that lead to global CNS failure. © 2005, Elsevier Inc.

I. Introduction

The first account of “brain water” can be ascribed to the ancient Egyptians some 2700 years ago (Breasted, 1930). During the Renaissance period, Andreas Versalius came up with a remarkably precise description of the cerebral ventricles and the choroid plexus (CP) in humans. He also calculated that the volume of “water-like fluid” that flows through “cavities” and “around the brain” accounts for approximately one-sixth of the total brain volume (see Clarke and Dewhurst, 1972). Studies of the human brain, using magnetic resonance imaging (MRI), revealed that the cerebrospinal fluid (CSF), or what Versalius called “water-like fluid,” encompasses 18% of the total brain volume (Luders et al., 2002). Given the lack of modern technologies, it is quite surprising how accurate Versalius was in his estimates of the CSF space.

For many decades the primary function of the CSF was thought to be the physical protection of the brain. It was not until the last 30–40 years that the growing body of evidence suggested a more active role of the CP-CSF system not only in the mature brain, but also during the development of...
the central nervous system (CNS). CSF is continuously produced by the four CPs of the third, fourth, and two lateral ventricles, and flows along the ventricular system and within the subarachnoid space (SAS), both distributing CSF-borne substances within the brain and clearing brain metabolites. In addition to its CSF secretory function, the choroidal epithelium synthesizes a large number of bioactive peptides (Chodobski and Szmydynger-Chodobska, 2001). Because the receptors for many of these peptides are expressed in choroidal tissue, it is possible that the peptides produced by the CP not only act on brain parenchymal cells, but also regulate the function of the CP itself. The CSF levels of various peptides and proteins produced by the CP change in several pathophysiological situations and in a number of CNS disorders, including brain injury, suggesting that the CP plays an important role in response to brain injury and, possibly, in the subsequent repair processes. The role of the CP in the transport and clearance of both endogenous molecules and xenobiotics, as well as in drug metabolism, has also been well documented (Miller et al., 2005). Therefore, CSF can no longer be considered to simply act as a “sink” for brain metabolites (Oldendorf and Davson, 1967). Rather, the CP-CSF system should be viewed as an active player in maintaining CNS homeostasis.

In this review, the authors will discuss the role of the CP-CSF system in the development of the CNS and analyze its functional importance in an adult and aging brain. The understanding of the normal physiology of the CP-CSF system and its malfunction or failure, as well as the appreciation of changes occurring in this system during normal aging, may open new avenues for designing effective treatment strategies for CNS disorders.

II. Fluid Compartments of the Brain

A. The Sources of Brain Interstitial Fluid (ISF) and CSF, Bulk Flow of ISF, and the Relationship Between CSF and ISF

There are two major compartments of extracellular fluid (ECF) in the brain: the ISF and the CSF (Fig. 1). It has been postulated that ISF is secreted by the endothelial cells of the brain microvessels into the perivascular space, from where it flows through the low-resistance pathways along the neuronal tracts and large-diameter blood vessels. The secretion rate of ISF in the rat brain has been estimated at 0.2 μl/min (Cserr et al., 1981), with the total ISF volume of 15–18% of the brain weight. In comparison to the flow of ISF, the CSF formation rate is quite rapid, amounting to 3.4 μl/min in the rat (Chodobski et al., 1998b). The majority of CSF is produced by the choroidal epithelium; however, some 10–30% of the total CSF flow is thought to be associated with the bulk flow of ISF. Even though the bulk flow of ISF was
discovered over 20 years ago (Cserr, 1984; Rosenberg et al., 1978, 1980), the controversy still exists as to what extent the flow of this fluid contributes to the total CSF production. Promoted by the pressure gradient built up across the ventricular system, CSF flows down the neuraxis eventually emptying into the SAS. Under normal conditions, there is a net movement of ISF from the brain parenchyma into the CSF. The ventricular CSF is separated from the surrounding brain tissue by the ependyma, whereas the CSF outside of the ventricles is separated from brain parenchyma by pial-glial lining. Both the ependyma and the pial-glial lining offer little hindrance to the convective flow of fluid and diffusional movement of CSF-borne substances into the brain parenchyma. CSF flows from the lateral ventricles into the third ventricle, and then continues its movement along the cerebral aqueduct and the fourth ventricle, eventually emptying into the subarachnoid space (SAS). CSF is reabsorbed from the SAS into the blood through the arachnoid villi/granulations protruding into the venous sinuses. Some CSF also drains along cranial nerves and spinal roots out to the lymphatics. Reprinted with permission from A. Chodobski and J. Szmydynger-Chodobska, 2001.

**Figure 1** Schematic diagram of the choroid plexus (CP)-cerebrospinal fluid (CSF) system. CPs are located in all four cerebral ventricles. The CPs are composed of tightly packed villous folds consisting of a single layer of cuboidal epithelial cells overlying a central core of highly vascularized stroma. The choroidal epithelium is continuous with ependymal lining, but it is morphologically and functionally different from the ependymal cells. The choroidal epithelial cells are joined by tight intercellular junctions. These epithelial tight junctions together with the arachnoid membrane form the blood-CSF barrier. The CPs are the major source of CSF; however, 10–30% of the total CSF production is represented by the bulk flow of interstitial fluid (ISF). Under normal conditions, there is a net movement of ISF from the brain parenchyma into the CSF. The ventricular CSF is separated from the surrounding brain tissue by the ependyma, whereas the CSF outside of the ventricles is separated from brain parenchyma by pial-glial lining. Both the ependyma and the pial-glial lining offer little hindrance to the convective flow of fluid and diffusional movement of CSF-borne substances into the brain parenchyma. CSF flows from the lateral ventricles into the third ventricle, and then continues its movement along the cerebral aqueduct and the fourth ventricle, eventually emptying into the subarachnoid space (SAS). CSF is reabsorbed from the SAS into the blood through the arachnoid villi/granulations protruding into the venous sinuses. Some CSF also drains along cranial nerves and spinal roots out to the lymphatics. Reprinted with permission from A. Chodobski and J. Szmydynger-Chodobska, 2001.
transmission in the brain (see following). However, the direction of ISF flow can be transiently reversed in response to changes in hydrostatic or osmotic pressure, allowing CSF-borne substances to enter the brain (Pullen et al., 1987; Rosenberg et al., 1978, 1980).

B. Volume Transmission

It has been postulated that both ISF and CSF play a key role in the so-called volume transmission (Agnati et al., 1995). The concept of volume transmission has been proposed to describe the chemical communication in the CNS involving both the short-distance (diffusional) and the long-range (convective—thanks to the continual secretion and flow of CSF and ISF) movement of signaling molecules within the ECF space of the brain. Thus, volume transmission complements the classical mode of intercellular communication involving synaptic and gap junction-mediated signaling. The ependymal lining of the cerebral ventricles and the pial-glial lining at the outer surface of the brain (Fig. 1) are permeable to the high-molecular-weight markers (Brightman and Reese, 1969), allowing for a free diffusional exchange between the CSF and the ISF. The penetration of brain parenchyma by CSF-borne molecules (e.g., peptides produced by the choroidal epithelium and released into the CSF) is, however, limited to the neuropil located immediately under the ependyma or the pial-glial lining (Ghersi-Egea et al., 1996; Proescholdt et al., 2000). Nevertheless, a considerable body of evidence has accumulated demonstrating that the biologically active substances administered into the CSF can exert significant physiological and behavioral effects that frequently require the activation of large and/or diverse populations of parenchymal cells. Despite intense research into this area, the underlying mechanisms of this “integrative” CSF function remain incompletely understood. It is possible that the biological effects of some CSF-borne peptides involve the receptor-mediated retrograde transport in neurons whose axonal processes are located near the ependymal or the pial-glial lining (Ferguson and Johnson, 1991; Ferguson et al., 1991; Mufson et al., 1999). Access to the deeper layers of brain parenchyma by CSF-borne substances may also be facilitated by the movement of these molecules along the perivascular Virchow-Robin spaces that are in contact with CSF (Agnati et al., 2005).

C. Protein Composition of the CSF

The amount of protein in the CSF is low (normally <0.5%) when compared to plasma, but the protein composition of this fluid is complex. These proteins may originate from several sources: exclusively from plasma, like
albumin; primarily from plasma, but are also with a significant proportion synthesized intrathecally, like soluble intercellular cell adhesion molecule 1 (sICAM1); primarily from the CP, like transthyretin (TTR); or primarily from brain parenchyma, like Tau protein (for review, see Reiber, 2001). The main protein fraction in normal CSF originates from plasma and is represented by albumin (Reiber and Peter, 2001). Approximately 20% of CSF proteins are derived predominantly from the brain, but they are hardly ever brain specific. For example, TTR in CSF predominantly originates from the CP, but this protein is also synthesized peripherally in the liver; in another example, the γ monomer of neuron-specific enolase (NSE) is not only a neuronal protein, but is additionally synthesized in erythrocytes and thrombocytes. The basic feature of predominantly brain-derived proteins is their higher concentration in the CSF compared to plasma, resulting in their net flux out of CSF, whereas for peripherally produced proteins there is a net flux into the CSF. Some CSF proteins that are both brain derived and produced by peripheral organs, if present at high plasma levels, may contribute a nonnegligible fraction to their CSF concentrations.

The differences between brain-derived and plasma-derived proteins are best characterized by the CSF/plasma concentration ratio and the so-called intrathecal fraction (IF). CSF/plasma concentration ratios for brain-derived proteins are relatively high (e.g., 1:1 for NSE to 34:1 for β-trace protein) compared to the CSF/plasma concentration ratios for plasma-derived proteins (e.g., 1:205 for albumin to 1:3400 for IgM). The calculated IF is very high for brain-derived proteins (e.g., 99% for Tau protein, NSE, S-100B, cystatin C, or β-trace protein), but <0.1% for the proteins that, under normal conditions, are exclusively plasma derived. Brain-derived proteins with a nonnegligible plasma-derived fraction in the CSF, such as TTR, sICAM1, and a soluble form of angiotensin converting enzyme (ACE), have intermediate CSF/plasma concentration ratios (1:18 to 1:190), with IFs ranging between 90% and 30%, respectively.

III. The CP-CSF System and the Development of the CNS

A. Development of the CP-CSF System

The formation of cerebral ventricles, the meninges, and the CPs takes place early during embryogenesis. The CP differentiates from the ependymal cells lining the ventricular walls and, in fact, is frequently considered to be a specialized cuboidal epithelium of ependymal lineage (Ek et al., 2005). The fourth ventricle CP develops first, followed by the CPs of both lateral ventricles and the third ventricle CP (Dziegielewska et al., 2001). The sequence of these events is quite uniform across mammalian species;
however, the timing of the appearance of individual CPs varies among the species and is most likely related to the length of gestation. Interestingly, the neural tube is filled with fluid before the CPs are formed, raising the question as to whether any CP-like cells capable of fluid secretion are present prior to the morphogenesis of the CP. Regardless of its source, the fluid filling the neural tube is rich in factors that are necessary for normal neurogenesis. For more information on embryogenesis and morphogenesis of the CP, the reader may refer to other reviews (Dziegielewska et al., 2001; Ek et al., 2005).

The CSF formation rate appears to increase gradually during ontogenesis (Evans et al., 1974; Holloway and Cassin, 1972; Johanson and Woodbury, 1974). In sheep, for example, a progressive increase in CSF production takes place both before and after birth (Evans et al., 1974). In rats, the maximum rate of brain growth is observed after birth (Dobbing and Sands, 1979). In these animals, the gradual increase in CSF formation during their postnatal development (Johanson and Woodbury, 1974) correlates well with postnatal morphological changes occurring in the choroidal epithelium (Keep and Jones, 1990; Keep et al., 1986) and with the maturation of the choroidal capability to transport K⁺ and Cl⁻ (Parmelee and Johanson, 1989; Preston et al., 1993). Blood flow to the CP, a limiting factor in the CSF production (Cserr, 1971), has also been found to increase gradually in rats after their birth (Szmydynger-Chodobska et al., 1994). These changes in blood flow to the CP likely reflect a progressive adjustment of the choroidal vasculature to steadily increasing secretory capabilities of the maturing choroidal epithelium.

In addition to its CSF secretory function, the choroidal epithelial cells form the physical and functional barrier between the blood and the CSF known as the blood-CSF barrier (BCSFB). The tight junctions between adjacent epithelial cells appear to be quite well developed in immature CP (Ek et al., 2005), suggesting that, in a growing brain, the properties of this epithelial barrier are largely similar to those typical of adult BCSFB. The apical surface area of the choroidal epithelium appears to be only two times smaller than that of the blood-brain barrier (BBB), suggesting that the BCSFB plays a much more important role in maintaining brain homeostasis than was previously thought (Keep and Jones, 1990).

CSF is not only important for the physical protection of a growing brain, but it also appears that the maintenance of sufficient CSF pressure within the ventricular system is essential for normal development of the CNS. Indeed, in an elegant study on chicken embryos, it was demonstrated that the slow drainage of the CSF from the ventricular system causes significant abnormalities in the neuronal organization of a developing brain (Desmond and Jacobson, 1977). Spina bifida, one of the most common malformations of human CNS resulting from the failure of fusion of the caudal neural tube,
has also been demonstrated to have serious consequences for the normal
development of the cerebral cortex (Miyan et al., 2001). In addition to
maintaining optimal hydrostatic pressure, circulating CSF exerts “nourish-
ing” effects on the developing brain by supplying critical growth factors and
other biologically active substances (see later discussion).

B. The Role of Peptides and Other Biologically Active Substances
   Either Synthesized in the CP or Transported Across the BCSFB in Brain Development

Both immature and adult CPs synthesize a large number of neuropeptides,
growth factors, and cytokines. In an embryonic brain, the CPs almost
completely fill the ventricular cavities whose size is disproportionately large
compared to the thin layer of neuroepithelium (Netsky and Shuangshoti,
1975). Therefore, the diffusional distances for CSF-borne bioactive sub-
stances (produced by the choroidal epithelium and released into the CSF
and/or transported from the blood into the CSF across the BCSFB) to their
putative targets in the developing neural tissue are much shorter than those
found in an adult brain. This raises the intriguing possibility that the
embryonic CP plays an active role in the development of the CNS.

1. Transthyretin

TTR, also referred to as prealbumin, is a carrier protein for thyroxine (T4),
the main hormone synthesized by the thyroid gland. The biologically active
principle of T4 is triiodothyronine (T3). The latter hormone is primarily
derived from the local deiodination of T4, which is mediated in the brain
by type II deiodinase (van Doorn et al., 1986). The mRNA levels and the
activity of this enzyme are tightly regulated in the CNS (Burmeister et al.,
1997) so that the T3 concentration in the brain is maintained at a relatively
stable level. An interest in TTR among developmental biologists has been
prompted by observations that the thyroid hormones are indispensable for
normal growth of the CNS (Anderson, 2001; Bernal and Nunez, 1995;
Oppenheimer and Schwartz, 1997). The liver and the CP have been identified
as two major sources of TTR. It has been noted, however, that transcriptional regulation of the TTR gene in the CP differs from that in the liver
(Yan et al., 1990). The choroidal epithelium, in which synthesis of TTR
begins at an early stage of brain development (Cavallaro et al., 1993), was
initially considered as the only source of this carrier protein in the CNS.
However, more recent studies have shown that, in addition to the CP, TTR
can be produced in the hippocampus, most likely by neurons, in response to
various experimental manipulations (Long et al., 2003; Stein and Johnson,
Interestingly, some peripherally produced TTR finds its way into the CNS (Terazaki et al., 2001), but the mechanisms by which blood-borne TTR enters the brain and the physiological significance of this phenomenon are presently unclear.

Using the isolated perfused CP and the primary choroidal epithelial cell cultures, researchers (Schreiber et al., 1990; Southwell et al., 1993) demonstrated that TTR is secreted into the CSF. Based on these findings, a model for T4 transport into the brain across the choroidal BCSFB was proposed. They theorized that newly synthesized TTR binds T4, either within the choroidal epithelium or in the CSF, immediately after its release into this fluid, and TTR-T4 complexes reach the brain parenchyma via the CSF pathways. An enzymatic conversion of T4 to T3 then occurs locally within neuropil. This hypothesis is, in part, supported by observations that extracellular markers injected into the lateral ventricle of the rat are rapidly distributed within the CSF space (Ghersi-Egea et al., 1996; Proescholdt et al., 2000). However, in another study (Dratman et al., 1991) in which the radiolabeled T4 was administered directly into the ventricular CSF, a markedly restricted distribution of the tracer within the brain parenchyma was observed. The latter finding indicates that transport across the BCSFB is not the major mechanism by which T4 is delivered into the CNS. This conclusion is also consistent with observations made in TTR knockout mice. These animals appear to be phenotypically normal, viable, and fertile (Episkopou et al., 1993). Research (Palha et al., 2000) shows that even though concentrations of T4 and T3 in the CP of TTR-deficient mice are significantly lower compared to wild-type controls, the levels of these hormones in the brains of TTR-null mice are normal. These results suggest that TTR is neither critical for T4 entry into the brain, nor is it necessary to maintain optimal T3 levels in the CNS. Although further studies will be needed to clarify the physiological significance of CP-derived TTR in the delivery and central homeostasis of thyroid hormones, it is important to note that TTR produced by the choroidal epithelium may have other important functions, such as the regulation of metabolism of β-amyloid in the CNS (see following). Using TTR knockout mice, it has also been demonstrated that TTR, most likely of CP origin, exerts significant behavioral effects (Sousa et al., 2004).

2. Insulin-Like Growth Factor 2

Insulin-like growth factor 2 (IGF2) is highly expressed in the CP and leptomeninges, even at early stages of mammalian development, and it continues to be synthesized by these tissues in adult brain (Bondy et al., 1992; Hynes et al., 1988; Logan et al., 1994). In contrast, the message for IGF2 has not been detected in either cells of neuroepithelial origin at any
stage of CNS development or in normal mature brain. Interestingly, in both humans and rodents, there is a biallelic expression of the \textit{IGF2} gene in the CP and leptomeninges, whereas in many other tissues, this gene is expressed only from the paternal allele (DeChiara \textit{et al.}, 1991; Ohlsson \textit{et al.}, 1994; Overall \textit{et al.}, 1997). The reason for the parental imprinting of the \textit{IGF2} gene is unclear, but it is possible that its biallelic expression in the CP and leptomeninges is necessary for normal development of the CNS. Studies in rats have demonstrated that prior to CP morphogenesis (E10), the message for IGF2 is abundant in the mesenchymal component of the CP primordium, whereas no IGF2 mRNA could be detected in the primordial CP epithelium (Cavallaro \textit{et al.}, 1993). The mesenchymal levels of IGF2 expression decrease during morphogenesis of the CP and IGF2 mRNA is absent in the stroma of adult CP. In contrast to the choroidal stroma, in differentiating CP epithelium, IGF2 mRNA gradually increases as embryogenesis progresses. These observations suggest that during early CNS development, mesenchyma-derived IGF2 acts to promote the differentiation of choroidal epithelial cells, whereas epithelium-derived IGF2 may be involved in the development of other parts of the brain and may also play a role in the normal functioning of adult CNS and/or repair after injury (see later discussion). This hypothesis is consistent with an early expression of receptors for this growth factor in both the CP and other brain areas (Bondy \textit{et al.}, 1992; Kar \textit{et al.}, 1993).

There are two types of insulin-like growth factor receptors (LeRoith \textit{et al.}, 1993; Sara and Hall, 1990). The type I receptor (IGF1R) has the highest affinity for insulin-like growth factor 1 (IGF1), but it also recognizes IGF2 and binds insulin at higher concentrations. The type II receptor (IGF2R) has a higher affinity for IGF2 compared to IGF1 and does not recognize insulin. The IGF1R mediates the mitogenic and neurotrophic effects of IGFs, and the binding of these ligands to IGF1R results in autophosphorylation of tyrosine residues in the intracellular part of this receptor (LeRoith \textit{et al.}, 1993; Sara and Hall, 1990). Interestingly, IGF1R is not only present in the developing CP, but is also highly expressed in the choroidal epithelium of adult animals (Nilsson \textit{et al.}, 1992), suggesting the autocrine/juxtacrine actions of IGF2 on mature CP. Because adult choroidal epithelial cells have a very slow turnover rate (McDonald and Green, 1988), it is unlikely that, under normal conditions, IGF2 has a mitogenic effect on choroidal epithelium. However, this growth factor may play a critical role in promoting a rapid recovery of choroidal tissue following the ischemic insult (Johanson \textit{et al.}, 2000). The IGF2R is structurally unrelated to the IGF1R and does not possess tyrosine kinase activity. The functional importance of IGF2R is not completely understood, but it is likely that this receptor plays a role in controlling the levels of IGFs in extracellular fluids by binding and subsequently degrading these growth factors (Haig and Graham, 1991).
Data obtained in primary cultures of choroidal epithelial cells (Holm et al., 1994; Nilsson et al., 1996) support the idea that IGF2 is secreted from the choroidal epithelium into CSF. In addition to IGF2, the CP produces, and most likely secretes, a number of insulin-like growth factor binding proteins (IGFBPs), with IGFBP2 and IGFBP4 being expressed at the highest levels (Holm et al., 1994; Stenvers et al., 1994; Walter et al., 1997). These proteins not only act as carriers for IGFs, but also play an important role in modulating the biological activity of these growth factors (Clemmons et al., 1993; Sara and Hall, 1990). Both IGF2 and IGFBP2 could be detected in myelinated nerve tracks in the brain, that is, in the areas that are remote from the site(s) of their synthesis (Logan et al., 1994). This and other studies (see later) thus provide evidence suggesting that CP-derived IGF2, together with its binding proteins, can act distally on their target cells in various areas of the CNS after being delivered via the CSF pathways.

3. Transforming Growth Factor-β Superfamily

The members of the superfamily of transforming growth factor-β (TGF-β) have been recognized as important regulators of various cell functions, including proliferation, differentiation, and survival. There are three isoforms of TGF-β: TGF-β1, TGF-β2, and TGF-β3. Only TGF-β3 is expressed in embryonic CP (Pelton et al., 1991), whereas all isoforms of TGF-β are expressed in the epithelial cells of an adult CP (Knuckey et al., 1996). The biological importance of CP-derived TGF-β during brain development is not completely understood, but it is likely that this growth factor plays a role in controlling the neuronal organization of developing CNS. Indeed, researchers (Chesnutt et al., 2004) using small interfering RNA to silence the expression of SMAD4, a critical element in TGF-β signaling, found in the chick embryo that the members of the TGF-β superfamily are essential for normal pattern formation and the specification of neural progenitor populations in the dorsal neural tube. TGF-β has also been shown to play an important role in both the induction and survival of dopaminergic neurons in the midbrain (Farkas et al., 2003). Further studies are clearly needed to more precisely define the role of CP-derived TGF-β in the developing CNS.

Bone morphogenetic proteins (BMPs) are another subfamily of proteins belonging to the TGF-β superfamily. Several members of this subfamily are present in the embryonic mouse brain, with BMP4, BMP5, BMP6, and BMP7 being expressed in the CP (Furuta et al., 1997). BMPs play an essential role in the development of CP, because in type I BMP receptor mutant mice, the lateral ventricle CPs are greatly reduced or fail to form (Hébert et al., 2002). Interestingly, the disruption of BMP signaling does not appear to affect the development of the rest of the telencephalon. BMP6 and BMP7 continue to be expressed in the adult CP (Charytoniuk et al., 2000).
These CP-derived BMPs may play a role in neuronal repair processes following ischemic brain injury (Charytoniuk et al., 2000).

Recently, a new member of the TGF-β superfamily, growth/differentiation factor 15 or macrophage-inhibiting cytokine 1 (GDF-15/MIC-1), has been cloned. High levels of mRNA for this growth factor have been found in the CP of both newborn (P0) and adult rats (Schober et al., 2001). The hypothesis based on the concept of volume transmission has proposed that GDF-15/MIC-1, following its release into the CSF, acts on developing neurons and/or glial cells in brain parenchyma. Again, further studies will be necessary to ascertain the function of this CP-derived protein in both the developing and mature CNS.

4. Fibroblast Growth Factors

Although several members of the family of fibroblast growth factors (FGFs) are expressed in the developing brain, only FGF7 (keratinocyte growth factor) and FGF2, also known as basic FGF, are expressed in the embryonic CP (Finch et al., 1995; Raballo et al., 2000). By comparison, four isoforms of FGF receptor, FGFR1–FGFR4, are present in immature choroidal tissue, with FGFR1, FGFR2, and FGFR4 being expressed on the epithelial cells and within the choroidal mesenchyma, and FGFR3 having nuclear localization (Reid and Ferretti, 2003). These observations suggest that CP-derived FGFs act as the autocrine and/or juxtacrine/paracrine regulators of CP development. At the same time, volume transmission may be involved in the regulation of CP growth by other members of the FGF family expressed in other parts of the developing brain. For instance, FGF8, originally identified as androgen-induced growth factor, is expressed in the commissural plate of the embryonic rodent brain and has been found to play an essential role in the normal development of the CP (Theil et al., 1999). Although direct evidence has yet to be established, it is possible that CP-derived FGF2 not only affects the development of the CP, but also controls the growth of other parts of the CNS. Indeed, Fgf2 knockout mice, though viable and fertile, exhibit significant abnormalities in the cytoarchitecture of the cerebral cortex (Ortega et al., 1998; Raballo et al., 2000).

5. CP-Derived Chemorepellents

Diffusible chemorepellents play a critical role in axon guidance during the development of the CNS. Studies (Hu, 1999; Nguyen-Ba-Charvet et al., 2004; Tamada and Murakami, 2004) have demonstrated that the CP has the ability to synthesize and release such chemorepellents, suggesting that this tissue can provide the guidance cues for growing axons. Two members of the Slit protein family of chemorepellents (Wong et al., 2002), SLIT2 and
SLIT3, and a secreted member of the semaphorin family (Raper, 2000), semaphorin 3F (SEMA3F), have been found to be expressed in the CP. Using explants of choroidal tissue, including those isolated from Slit2−/− mice, as well as employing COS cells expressing SLIT2, several groups have demonstrated that CP-derived SLIT2 repels the precursors of olfactory interneurons (Hu, 1999; Nguyen-Ba-Charvet et al., 2004; Tamada and Murakami, 2004). The repellent activity of the CP toward olfactory bulb axons was attenuated in the presence of the soluble form of Roundabout (Robo), a receptor for the Slit proteins (Tamada and Murakami, 2004). By comparison, CP-derived SEMA3F has been demonstrated to repel the axons from the epithalamic and hippocampal explants obtained from the embryonic rat brain (Tamada and Murakami, 2004). When the soluble form of neuropilin 2 (NRP2), a receptor for SEMA3F, was included in the assays, the repellent activity of the CP toward the epithalamic and hippocampal axons was reduced. These observations suggest that CP-derived SLIT2 and SEMA3F may control axonal growth in various regions of the developing brain. In this context, it is important to note that in their axon guidance actions, the chemorepellents produced by the CP may interact with other CP-derived growth factors. Indeed, NRP2 and neuropilin 1 (NRP1) do not only function as the receptors for semaphorins (Raper, 2000), but they also bind vascular endothelial growth factor (VEGF) that is highly expressed in the choroidal epithelium (Chodobski et al., 2003). A study has shown that the biological effects of semaphorin 3A (SEMA3A), a chemorepellent binding to NRP1, are antagonized by VEGF (Bagnard et al., 2001). These authors have also demonstrated that the SEMA3A actions require the presence of type I VEGF receptor. These results indicate that axonal guidance involves specific interactions at both the ligand and receptor levels.

6. Retinoic Acid

Retinoic acid (RA), an active derivative of retinol (vitamin A), is essential for normal development of the CNS. It plays an important role in antero-posterior and dorsoventral patterning of neuronal differentiation, and its major sites of action are the hindbrain and the anterior part of the spinal cord (Maden, 2002). RA also controls the development of interneurons and motor neurons along the dorsoventral axis. Both RA overexposure and retinol deficiency cause major malformations of all hindbrain structures, frequently resulting in hydrocephalus. During the process of neuronal differentiation, RA regulates the expression of a large number of genes by binding to two classes of its receptors that operate as ligand-activated transcription factors (Bastien and Rochette-Egly, 2004). Two classes of enzymes, the alcohol dehydrogenases (ADHs) and the retinaldehyde dehydrogenases (RALDHs), are involved in RA synthesis. RA synthesis is also facilitated
by the cellular retinol-binding proteins (CRBPs) that sequester retinol and present it to specific dehydrogenases (Ottonello et al., 1993).

The CP has the ability to produce RA. This tissue expresses ADHs, though conflicting data have been reported with regard to which members of the ADH family are present in the CP (Galter et al., 2003; Martinez et al., 2001). In addition, the CP expresses RALDH2 (also known as the A2 member of the aldehyde dehydrogenase 1 family; ALDH1A2) and CRBP1, with the latter protein appearing to enhance the enzymatic activity of RALDH2 in the choroidal tissue (Ruberte et al., 1993; Yamamoto et al., 1998). The fourth ventricle CP is closely associated with the cerebellum throughout its development and into the mature CNS. Unlike the developing CP, which is highly active in producing RA, the growing cerebellum has a rather limited capability to synthesize RA (Yamamoto et al., 1996). Normal development of the cerebellum is extremely sensitive to an imbalance in the levels of retinoids, as, for example, an excess of RA has been found to have potent teratogenic effects on this part of the brain (McCaffery et al., 2003). Based on these observations, it has been proposed that the fourth ventricle CP plays a key role in the development of the cerebellum by being an important source of RA for this hindbrain structure (Yamamoto et al., 1996). These authors have found biphasic changes in the choroidal activity of RALDH2 in both mice and rats, with the first peak of enzymatic RALDH2 activity occurring at E18, followed by the second peak at days 6–8 of postnatal development. These changes in the choroidal RALDH2 activity correlate well with distinct developmental events in the cerebellum. The first peak in RALDH2 activity observed at an embryonic stage of CNS development coincides with the somatic and axonal differentiation of Purkinje cells, whereas the postnatal peak is paralleled by the dendritic arborization of Purkinje cells and differentiation of granule cells. Interestingly, RA is not only critical for the development of the cerebellum, but it also appears to play an important role in the growth of the CP, given that the insufficient dietary intake of vitamin A affects the development of choroidal tissue (see discussion in Ruberte et al., 1993). Although the enzymatic activity of RALDH2 decreases substantially in the choroidal tissue of the mature brain, an adult CP still maintains the ability to produce RA. The physiological significance of this choroidal function remains unclear, however.

7. **Leptin**

In previous sections, we have analyzed the ability of the choroidal epithelium to produce various bioactive peptides and RA, and discussed how these CP-derived substances may affect brain development. Here, the possible role of the BCSFB-mediated transport of leptin (LEP) in the development of the CNS will be discussed. LEP, the product of the obesity (ob) gene, was
discovered through positional cloning (Zhang et al., 1994). This hormone is mainly synthesized by white adipose tissue and its serum levels depend on the percentage of body fat (Considine et al., 1996; Maffei et al., 1995). LEP plays a critical role in the regulation of energy balance of the body by acting on several groups of hypothalamic neurons (Ahima and Flier, 2000). The LEP receptor (LEPR) has been identified through expression cloning and found to be present at exceptionally high levels in the CP (Tartaglia et al., 1995). Later on, it was determined, however, that the choroidal LEP receptor is a short isoform of LEPR that has a limited signaling capability compared to the long isoform expressed in the hypothalamus (Bjørbæk et al., 1997; Ghilardi et al., 1996). It has therefore been proposed that the choroidal LEPR plays a role in the receptor-mediated transport of LEP from the blood into the CSF. This idea is supported by studies in which in situ rat brain perfusion and perfused sheep CP models were used to demonstrate the transport of radioiodinated LEP across the BCSFB (Thomas et al., 2001; Zlokovic et al., 2000). Reduction in the capacity of the choroidal LEP transport and/or impairment of the transport of this hormone across the BBB has been proposed to cause LEP resistance, leading to obesity. Further discussion on this subject will be presented in a later section.

It has been recognized for some time that obese Leptinob/ob mice that do not produce functional LEP have several abnormalities of the CNS, such as reduced brain weight and brain DNA content, as well as altered dendritic orientation and abnormal myelination (Ahima et al., 1999; Bereiter and Jeanreneaud, 1979, 1980; Steppan and Swick, 1999; Sena et al., 1985; van der Kroon and Speijers, 1979). It has also been shown that in these animals, the total brain protein content is lower compared to wild-type littermate mice (Ahima et al., 1999). Specific analysis of several neuronal and glial proteins has demonstrated that the levels of expression of various synaptic proteins, such as synaptosome-associated protein of 25 kDa (SNAP-25), syntaxin 1 (STX1), and synaptobrevin, are reduced in the cerebral cortex, hippocampus, and hypothalamus of Lepob/ob mice. Similar results were obtained in obese diabetic (db) Leprdb/db mice, in which obesity results from an abnormal splicing of LEPR (Lee et al., 1996). These changes in expression of synaptic proteins were associated with either LEP deficiency or impaired LEP signaling and not with obesity itself, as they were not observed in obese, LEP-resistant agouti (A/y/a) mice. When immature Lepob/ob mice had received daily intraperitoneal injections of recombinant LEP, researchers observed that the brain levels of expression of SNAP-25 and STX1 were restored. LEP replacement therapy has also been found to improve the locomotor activity of Lepob/ob mice, which did not appear to be secondary to the loss of body weight resulting from LEP administration, but was instead most likely mediated by LEP itself (Ahima et al., 1999). These observations strongly suggest that LEP plays an important role in the
development of the CNS. The widespread expression of the long form of LEPR observed in various structures of the embryonic rat brain (Udagawa et al., 2000) is consistent with the preceding hypothesis.

In a recent study, scientists investigated the role of LEP in the development of neuronal projections from the arcuate nucleus (ARC), one of the major hypothalamic areas involved in the regulation of food intake (Bouret et al., 2004). These authors have shown that in Lep<sup>ob/ob</sup> mice, neuronal projections from the ARC are permanently disrupted and that LEP replacement in adult mutant mice does not reverse this anatomical defect. Interestingly, there is a prominent surge in serum LEP levels in rodents during their first 2 weeks of postnatal development (Ahima et al., 1998; Morash et al., 2001). Accordingly, in neonatal Lep<sup>ob/ob</sup> mice treated with recombinant LEP it was found that, unlike adult mutants, immature Lep<sup>ob/ob</sup> mice respond to exogenous LEP with normal development of ARC projections (Bouret et al., 2004). LEP has also been shown to induce neurite outgrowth from the ARC in organotypic cultures of this hypothalamic nucleus obtained from P6 wild-type mice. These findings indicate that LEP is a critical factor in the development of hypothalamic neuronal pathways involved in the control of energy balance. It is important to note, however, that LEP may not only exert direct effects on immature neurons, but may also influence the development of the CNS by regulating the levels of other hormones, such as glucocorticoids (Ahima et al., 1999), that are known to affect brain development (Matthews, 2000).

Although it is likely that circulating LEP is transported into the brain during development, findings suggest that this protein can also be synthesized centrally in areas such as the hypothalamus, cerebral cortex, and cerebellum (Morash et al., 1999, 2001). Further studies will be needed to clarify the physiological importance of this central LEP synthesis for both immature and adult CNS.

**IV. The CP-CSF System in Adulthood**

In the previous sections, the role of CSF-borne substances and the involvement of the CSF pathways in the development of the brain was discussed. In this section, the focus will be on transport/clearance properties of the mature BCSFB and the possible role of the CP-CSF system in CNS injury.

**A. Transport Systems in the CP**

The exchange processes at the choroidal BCSFB are tightly controlled and involve complex regulatory mechanisms. Choroidal epithelial cells are equipped with a number of transporters that are localized to both the apical
1. The CP-CSF System

and basolateral membranes. This polarized distribution of transport systems is essential for the bidirectional movement of various substances across the choroidal epithelial barrier.

1. Transport of Glucose and Amino Acids

The Na\(^+\)-independent glucose transporter, GLUT1, is expressed exclusively on the basolateral membrane of choroidal epithelial cells (Kumagai et al., 1994). The transport of glucose across the BCSFB was studied by researchers (Deane and Segal, 1985) who used a model of the perfused sheep CP. These authors estimated the concentration of glucose in the newly formed CSF based on the rate of CSF secretion and the net flux of this sugar across the choroidal epithelium. The concentration of glucose in this fluid was found to be 45–60% of that in plasma, suggesting that the low glucose levels observed in bulk CSF are related to the entry process and not to the cerebral metabolism of this sugar.

An uptake of amino acids across the apical (CSF-facing) membrane of choroidal epithelium was initially demonstrated using an in vitro preparation of the CP (Caruthers and Lorenzo, 1974) and an in vivo ventriculo-cisternal perfusion technique (Davson et al., 1982). The existence of an apical, Na\(^+\)-dependent uptake of small neutral and charged amino acids was later confirmed by other researchers who employed either primary cultures of choroidal epithelial cells (Villalobos et al., 1997) or conditionally immortalized choroidal epithelial cells (Kitazawa et al., 2001; Terasaki and Hosoya, 2001). Studies employing the perfused sheep CP have shown that the uptake of amino acids across the opposite, basolateral membrane of choroidal epithelium is strictly equilibrative and mediated by the L-transport system (for large, neutral, and branched amino acids) and by the ASC system (for small, neutral amino acids) (Preston and Segal, 1990). Such distribution of amino acid transporters (equilibrative transporters in the basolateral membrane and concentrative transporters in the apical membrane) allows for the maintenance of a steep amino acid gradient between the CSF and the plasma, which may play a role in the removal of amino acids having neurotransmitter activities, such as glycine, from the CSF.

2. Transport of Nucleosides

Another group of transporters that are present in the choroidal epithelium are nucleoside transporters (for review on this topic, see Redzic, 2005). It appears that the distribution of nucleoside transporters in the choroidal epithelium is polarized and that this polarization is essential for the clearance of nucleosides from CSF. Studies (Wu et al., 1992, 1994) demonstrated that, in the rabbit CP, nucleosides are transported across the apical membrane
against the concentration gradient and in the presence of inwardly directed Na\(^+\) gradient. These authors have also provided the functional evidence that in the choroidal tissue, both purine and pyrimidine nucleosides are substrates for a single Na\(^+\)-nucleoside cotransport system, later designated the cib (concentrative, insensitive to NBTI, broad specificity) system. The cib transport system is now known to be represented by the transporter CNT3 (Gray et al., 2004; Ritzel et al., 2001). Studies in which an in situ perfused ovine CP model was used provided functional evidence that the uptake of purine nucleosides across the basolateral membrane of the choroidal epithelium is strictly equilibrative (Redzic et al., 1997). An analysis of adenosine uptake in primary cultures of rat choroidal epithelial cells confirmed that the distribution of nucleoside transporters is polarized, with the concentrative transport occurring exclusively across the apical (CSF-facing) membrane (Redzic et al., 2005). It is thus possible that the concentrative transporters expressed in the rat choroidal epithelial cells, rCNT2 (detected at both the mRNA and protein level) and rCNT3 (detected at the mRNA level), are confined to the apical membrane of choroidal epithelium. In contrast, the equilibrative transport, which is presumably mediated by the equilibrative nucleoside transporter 1 (rENT1), was only detectable across the basolateral membrane of choroidal cells (Redzic et al., 2005). This pattern of distribution of nucleoside transporters, together with the well-known rapid metabolism of adenosine within the choroidal epithelium (Pardridge et al., 1994; Redzic et al., 1997), suggests that CP plays a key role in both preventing circulating adenosine from entering into the CSF and in removing this nucleoside from CSF. This aspect of choroidal function may be critical for central signaling, given that adenosine can act as a neuromodulator.

3. Removal of Xenobiotics

An important aspect of the BCSFB function is protection of the brain from toxins and xenobiotics. This subject has been discussed in an excellent review (Miller et al., 2005). The choroidal epithelium expresses a number of transport proteins involved in the eflux of CSF-borne lipophilic compounds, such as etoposide and vinca alkaloids, and organic anions, such as p-aminohippurate, benzylpenicillin, and cimetidine. Pharmacokinetic and immunohistochemical studies have demonstrated the presence of the multidrug resistance (MDR) gene product MDR1 P-glycoprotein (Pgp) and the multidrug resistance-associated protein 1 (MRP1) in the choroidal epithelium (Rao et al., 1999; Wijnholds et al., 2000). Studies performed on mutant mice that lacked MRP1 have shown that their CSF levels of etoposide following intravenous (IV) administration of this compound are 10-fold higher than those observed in mice expressing MRP1 (Wijnholds et al., 2000). A considerable blood-to-CSF concentration gradient across the choroidal epithelium has been...
found in humans after peripheral infusion of $^{99m}$Tc-sestamibi, a membrane-permeant radiopharmaceutical whose transport is mediated by both Pgp and MRP (Rao et al., 1999). It has also been reported that an efficient efflux system for organic anions exists in the CP (Nagata et al., 2002). Using Western blotting and immunohistochemistry, these authors determined that the organic ion transporter (OAT) 3, but not OAT1, is expressed in the rat CP and that OAT3 is localized to the apical (CSF-facing) membrane of the choroid epithelial cells.

4. Transport of Peptides

a. Leptin. The effect of LEP on CNS development has been previously mentioned. In an adult organism, one of the key functions of this hormone is the maintenance of energy balance (Ahima and Flier, 2000). Convincing evidence has been provided that circulating LEP is transported across the BCSFB (Thomas et al., 2001; Zlokovic et al., 2000). However, the short isoforms of LEPR, thought to be involved in the transport of LEP into the brain, are also expressed in brain microvessels (Hileman et al., 2002). Consistent with these observations, LEP has also been found to cross the BBB (Banks et al., 1996, 2000; Zlokovic et al., 2000), and the importance of LEP transport across the BBB versus BCSFB is presently a matter of debate. (The reader will find a more detailed discussion of this subject in Chodobski et al., 2005.) Although the rodent models of obesity, such as Lepr$^{ob/ob}$ and Lepr$^{db/db}$ mice, are commonly used to study this disease, their genetic equivalents are rarely observed in humans (O’Rahilly et al., 2003). Rather, the frequent finding in obese individuals is elevated LEP concentrations in serum (Considine et al., 1996; Maffei et al., 1995). It has therefore been proposed that obesity in humans is associated with LEP resistance caused by defective LEP transport into the brain. This hypothesis is supported by a number of clinical and animal studies (Banks and Farell, 2003; Banks et al., 1999; Caro et al., 1996; Hileman et al., 2002); however, the mechanisms underlying this defect in LEP signaling are not completely understood. In this context, it is important to note that LEP resistance may also involve defective signal transduction in the hypothalamic LEPR (El-Haschimi et al., 2000). Further studies are likely to enhance our insight into LEP resistance, thus facilitating the identification of new potential targets for the treatment of obesity.

b. Prolactin. Prolactin (PRL), a hormone synthesized and secreted by the anterior pituitary, is commonly known for its involvement in mammary gland development and lactation. Interestingly, the amino acid sequence of PRL shows similarity with two other hormones: growth hormone (GH) and placental lactogen (PL). Because of their structural homology and
a similarity of many biological features, these three proteins are called the PRL/GH/PL family. Recently, these hormones were linked to a more extended group of proteins referred to as hematopoietic cytokines (Goffin et al., 2002).

PRL elicits a variety of biological responses in different target tissues by binding to its cognate receptor PRLR. Several isoforms of PRLR have been identified, including short, intermediate, and long isoforms, all of which belong to the class I cytokine receptor family (Clevenger and Kline, 2001; Goffin and Kelly, 1997). In addition to PRL, the PRLR binds two other ligands, PL and GH (Goffin et al., 1996), which complicates the understanding of the biological effects produced by PRL.

Both radioligand binding analysis and in situ hybridization histochemistry have demonstrated a particularly high concentration of PRLR in the CP (Brooks et al., 1992; Lai et al., 1992). Consistent with these findings, research (Walsh et al., 1978) showed that, following its IV infusion, $^{125}$I-PRL heavily labeled the CP, whereas the cerebral vasculature was free of radioiodinated PRL. Based on these observations, the authors suggested that circulating PRL is transported into the brain across the BCSFB. This conclusion is supported by other studies from the same laboratory (Walsh et al., 1987) showing the presence of a saturable transport of PRL from the blood to the CSF. Further research, however, will be needed to determine the physiological significance of PRL transport across the BCSFB.

c. Clearance of CSF-Borne Oligopeptides. The choroidal epithelium is equipped with the enzymatic and peptide-transport systems that play important roles in the processing/degradation of CSF-borne peptides and the clearance of peptide degradation products. Many peptidases are present in the CP, with some of these enzymes (e.g., ACE and neprilysin) being highly expressed on the apical membrane of choroidal epithelial cells (summarized in Smith et al., 2004). Because of the large apical surface area of choroidal epithelium (previously discussed), these enzymes are in contact with a considerable amount of bulk CSF. Consequently, the choroidal peptidases are likely to play a significant role in the processing and degradation of CSF-borne peptides.

The physiological significance, substrate specificity, and transport kinetics of oligopeptide transporters present in the choroidal BCSFB have been reviewed (Smith et al., 2004). Two oligopeptide transporters, each being a member of a separate family of transporters, have been shown to be expressed in the CP. The first transporter, PEPT2 (Berger and Hediger, 1999), is responsible for the symport of dipeptides and tripeptides along an inwardly directed proton gradient. Functional and immunocytochemical experiments performed in primary cultures of rat choroidal epithelial cells have shown that PEPT2 is expressed apically in choroidal cells and mediates
the accumulation of a model dipeptide, glycylsarcosine, across the apical membrane of choroidal epithelium (Shu et al., 2002). These results suggest that PEPT2 plays a role in the clearance of oligopeptides and endogenous peptidomimetics from CSF. Another oligopeptide transporter identified to be expressed in the CP, though not yet functionally characterized in this tissue, is peptide/histidine transporter PTH1 (Yamashita et al., 1997). Similar to PEPT2, PTH1 transports dipeptides and tripeptides; however, PTH1 is also able to transport the amino acid L-histidine. Further studies are needed to define the functional importance of this choroidal transporter.

B. The Role of the CP-CSF System in CNS Injury

It has been demonstrated that in various types of brain injury, such as traumatic brain injury, ischemia, and subarachnoid hemorrhage (SAH), the CSF levels of several growth factors are elevated. The source(s) of these factors is still a matter of debate. However, considering the fact that transcripts for many growth factors have been identified in the CP (Chodobski and Szmydynger-Chodobska, 2001), it is likely that they originate, at least in part, from the CP. Studies by several groups (Borlongan et al., 2004a,b; Ide et al., 2001; Matsumoto et al., 2005) demonstrated that transplantation of choroidal cells to both traumatized spinal cord and ischemic brain have significant neuroregenerative and neuroprotective effects. Protection of striatal cholinergic neurons by choroidal grafting in a rodent model of Huntington’s disease has also been shown (Borlongan et al., 2004c). Furthermore, in various in vitro assays, both neuronal survival and neurite outgrowth have been found to be promoted by conditioned media from choroidal cultures or by the coculturing of choroidal epithelial cells with neurons (Borlongan et al., 2004a; Chakrabortty et al., 2000; Kimura et al., 2004). However, the nature of biologically active factors produced by the choroidal epithelium in response to injury and the mechanisms underlying the beneficial effects of CP grafting are not fully understood. In the following paragraphs, the possible roles of selected, CP-derived growth factors in brain injury will be analyzed.

1. Insulin-Like Growth Factor 2

The idea that an increase in CSF concentration of various growth factors observed after brain injury is a result of their augmented production by choroidal epithelium is supported by the study of Walter et al. (1999). These authors reported that after a localized brain injury, there was a transient increase in IGF2 concentration in the CSF that peaked at 7 days post-injury. These changes in the CSF IGF2 level were paralleled by increased
concentrations of immunoreactive IGF2 in the affected neuropil. Because the message for IGF2 was not increased in any parenchymal cells until later after the injury, it is highly likely that the IGF2 protein detected in the injured parenchyma is of CP/leptomeningeal origin. Interestingly, in the chronic phase (7–14 days after injury), the levels of IGF2 in the CSF declined, and this growth factor appeared to be predominantly synthesized by astrocytes located in the injured parenchyma. These observations suggest the existence of two major sources of IGF2 in the injured brain, with distinct, time-dependent synthetic activities. In the acute post-injury phase, IGF2 appears to be largely produced by the choroidal epithelium and leptomeninges, exerting endocrine-like effects on its target cells in both the injured neuropil and other unaffected areas of the brain. However, during the late post-injury period, the production and, possibly, biological actions of IGF2 are mainly confined to the injured parenchyma.

2. Transforming Growth Factor-β

As discussed above, TGF-β plays an important role in promoting neuronal survival. For example, studies in rodents have demonstrated that the intracerebroventricular (ICV) administration of a moderate dose (4 ng) of recombinant TGF-β1 one hour prior to the induction of transient forebrain ischemia has a significant neuroprotective effect on pyramidal neurons in the CA1 hippocampal region (Henrich-Noack et al., 1996). Interestingly, dose of TGF-β1 both approximately 10 times lower or higher did not affect neuronal survival in the CA1 region. Using a similar rat model of transient forebrain ischemia, researchers (Knuckey et al., 1996) showed an increase in the message for all three isoforms of TGF-β in the CP at 1–2 days after the insult. Increased choroidal expression of TGF-β1 in other models of brain injury, such as hypoxia-ischemia and localized cerebral injury, has also been observed (Klempt et al., 1992; Logan et al., 1992).

Clinical studies of patients with SAH have demonstrated biphasic changes in the concentration of TGF-β1 in the CSF (Flood et al., 2001). The first peak in TGF-β1 levels (1–2 days post-SAH) was associated with the disruption of the BBB, whereas the second peak (9–10 days post-SAH) was not. Researchers have attributed the first peak to the release of TGF-β1 from platelets, a rich source of this growth factor, whereas the second peak has been suggested to result from the central production of TGF-β1 in areas, such as the choroidal epithelium (Flood et al., 2001). This conclusion was supported by the observations that in CPs from SAH patients collected at 10–12 days after SAH, TGF-β1 was expressed at much higher levels than in the choroidal tissues obtained from control subjects. Based on these observations, it is tempting to speculate that in response to injury, larger amounts of TGF-β are secreted from the choroidal epithelium into the CSF, from which
this growth factor is transported to its parenchymal target cells to exert neuroprotective effects. Further studies are needed to test this hypothesis.

### 3. Fibroblast Growth Factor 2

Originally known as a strong mitogenic and angiogenic factor, FGF2 has been gaining attention as a growth factor with neurotrophic properties (Abe and Saito, 2001). FGF2 has also been shown to have the ability to modulate synaptic transmission. In an adult CP, the mRNA and protein for both FGF2 and its receptor, FGFR1, have been identified (Fuxe et al., 1996; Gonzalez et al., 1995). The receptors for FGF2 are expressed apically on the choroidal epithelium (Szmydynger-Chodobska et al., 2002) and their activation may play a role in the FGF2-mediated inhibition of CSF formation (Hakvoort and Johanson, 2000; Johanson et al., 1999b). Another study (Mufson et al., 1999) demonstrated a retrograde neuronal transport of FGF2 from the ventricular CSF, which was suggested by these authors to have important functional implications for the treatment of neurological disorders. This idea is supported by observations that FGF2 administered into the cerebral ventricles has a significant neuroprotective effect in focal cerebral ischemia in rodents (Koketsu et al., 1994; Ma et al., 2001). Intracerebroventricular infusion of the recombinant FGF2 has also been found to stimulate the differentiation of progenitor cells in the subventricular zone (SVZ) into neurons in both young adult and aged rodents (Jin et al., 2003; Kuhn et al., 1997). Considering the anatomical location of the SVZ and its proximity to the CSF, one can speculate that CP-derived FGF2 likely promotes neurogenesis in the SVZ. However, FGF2 may also exert adverse effects on neurons. For example, high doses of FGF2 infused into the cerebral ventricles have been found to promote neuronal apoptosis in the caudate-putamen (Chodobski et al., 1998a), possibly through the caspase-dependent mechanisms and downregulation of BCL2 expression (Burchill and Westwood, 2002; Wang et al., 1998). Elevated levels of FGF2 in the CSF have been observed in various CNS disorders, including moyamoya syndrome, Chiari malformation, and hydrocephalus (Malek et al., 1997), suggesting a broad range of biological actions of this peptide in the CNS. These putative actions of FGF2 await further investigation.

### C. Possible Sources of Stem Cells in the CNS and Their Relation to the CP-CSF System

Historically, the possibility of neurogenesis in adult mammalian brain has been rejected. Only recently has both the turnover of neuronal cells in the adult CNS been shown and a pool of pluripotent stem cells been identified in
the SVZ, just under the ependymal lining of the lateral ventricles (Lois and Alvarez-Buylla, 1993; see also review by Galli et al., 2003). It has been demonstrated that these SVZ cells, when maintained under appropriate conditions, may undergo differentiation into neurons or astrocytes (Galli et al., 2003; Lois and Alvarez-Buylla, 1993). Researchers (Doetsch et al., 1997) have described the topographical organization of the SVZ and identified three distinct types of SVZ cells: type A, with an ultrastructure of migrating neuronal precursor cells; type B (B1 and B2), with characteristics of astrocytes; and type C, with ultrastructural characteristics of immature cells. Interestingly, type B cells frequently have direct contact with CSF by protruding between the ependymal cells (Alvarez-Buylla and Garcia-Verdugo, 2002). Although our knowledge about the mechanisms regulating the differentiation, migration, and integration of new neuronal cells in adult CNS remains incomplete, the close location of pluripotent SVZ cells to the CSF space suggests that CP-derived growth factors, such as FGF2 (see previous discussion) and heparin-binding epidermal growth factor-like growth factor (Mishima et al., 1996), influence the fate of these cells (Jin et al., 2003).

In 2001 intriguing work was reported (Ide et al., 2001). It was demonstrated that the fourth ventricle CP excised from the brain of an adult rat and grafted into the dorsal funiculus of rat spinal cord can promote axonal growth in the host. Later that year, the same group (Kitada et al., 2001) showed that the choroidal epithelial cells harvested from adult mice, cultured for 4–6 weeks, and then grafted into the prelesioned spinal cord of the same species have the ability to differentiate into astrocytes. Within 1 week following the grafting of choroidal cells, some transplanted cells were found to stain positively for glial fibrillary acidic protein (GFAP), an astrocytic marker. After 2 weeks, these GFAP-positive cells demonstrated the morphological characteristics of astrocytes and appeared to be fully integrated in the host tissue. Although in their spinal cord injury model, Kitada et al. (2001) were not able to show that the transplanted choroidal cells differentiate into neurons, another study (Li et al., 2002) suggests that the choroidal epithelium has the potential to differentiate into neurons after focal cerebral ischemia. In this latter study, a small population of bromodeoxyuridine-positive cells, presumed to represent proliferating cells, was found to costain for the neuronal marker NeuN in the lateral ventricle CP ipsilateral to the injured hemisphere. Further studies will be needed to confirm these preliminary observations and evaluate their physiological significance.

V. Senescence of the CP-CSF System

Descriptions of age-related changes to the CP-ventricular system have drawn many parallels with CNS pathologies. While it is clear that certain aspects of CP-CSF senescence are not in themselves a disease state, the links between
the aging system and two pathologies, normal pressure hydrocephalus (NPH) and Alzheimer’s disease (AD), have been outlined in the following.

A. Aging Parallels with Hydrocephalus?

1. Ventriculomegaly and CSF Drainage

One of the first changes to the CSF axis in later life is an increase in CSF volume, particularly ventricular volume that increases by 25–30% in 50- to 80-year-old people compared to 20- to 40-year olds (Pfefferbaum et al., 1994), but also SAS volume (Narr et al., 2003). As a proportion of total intracranial space, the CSF occupies only 7–9% of intracranial volume in adolescence, but starts to increase soon after the second decade of life, reaching 20–33% of intracranial volume by age 71–80 (Courchesne et al., 2000). These changes are exaggerated in neuropathological conditions, including schizophrenia (Narr et al., 2003) and dementia, but in healthy aging, elevated intracranial volume is largely assumed to be secondary to brain atrophy and in one study, total brain volume was smaller in 71- to 80-year olds than in a healthy 2- to 3-year-old child (Courchesne et al., 2000). Grey matter volume changes are prominent and this volume diminishes by around 5% per decade after adolescence; white matter is relatively preserved but still falls by 13% between the fourth and eighth decades.

Accompanying the increased CSF volume, there is evidence for increased resistance to CSF drainage in healthy middle and later life (Albeck et al., 1998), probably as a result of a combination of calcification of the arachnoid villi, thickening of the arachnoid membrane (Bellur et al., 1980), and central vascular hypertension (Rubenstein, 1998). It is striking that the gross changes seen in the aging CSF system resemble changes in NPH characterized by increased intracranial CSF volume, but which, in NPH, is thought to be secondary to increased outflow resistance (R_out) rather than brain atrophy (Boon et al., 1998; Borgesen et al., 1982). Prevalence of NPH increases significantly with age and the pathophysiology is largely unknown (Eide et al., 2003), but risk factors include previous cerebral diseases or trauma, such as meningitis and hemorrhage (Silverberg et al., 2003), and cerebrovascular disease (Boon et al., 1999). Among patients with NPH, R_out significantly increases with age (Czosnyka et al., 2001; Eide et al., 2003), as it does in healthy subjects.

2. CSF Secretion

Given the “closed” nature of the CSF circulatory system, any increase in resistance to drainage, R_out, would be expected to elevate intracranial pressure (ICP), following the relationship: ICP = R_out × CSF secretion.
rate × sinus pressure (Davson et al., 1987), but no correlation between ICP and age is seen in healthy subjects or NPH patients (Czosnyka et al., 2001; Eide et al., 2003). Indeed, for NPH, ICP is usually within the normal range, at least during the day (Eide et al., 2003). An explanation may lie in the downregulation of CSF secretion rate in both healthy aging and NPH. Comparing CSF secretion rates in patients with NPH, Parkinson’s disease (PD), AD, and acute hydrocephalus, observers (Silveberg et al., 2003) found that NPH patients had the lowest secretion rates, at just over 0.2 ml/min, comparable to AD patients (mean age 72; Silverberg et al., 2001), whereas secretion rates in PD (mean age 69; Silverberg et al., 2001) and acute hydrocephalic patients were almost double NPH levels (Silverberg et al., 2002). Decreased CSF production rate in NPH has also been seen in studies (Czosnyka et al., 2001) in humans and in animal models of chronic hydrocephalus (Marlin et al., 1978; Sahar et al., 1971). In animal models, ion transport, fundamental to CSF secretion, which could be analyzed and reduced transfer of both Na⁺ (Marlin et al., 1978) and Cl⁻ (Knuckey et al., 1993) seems to underlie diminished CSF secretion, at least in kaolin-hydrocephalus models. Measurements of CSF secretion in aging are less consistent in humans; an early study using the invasive Masserman technique suggested that secretion rates halved from 0.4 to 0.2 ml/min with age, comparing two cohorts averaging 29 and 77 years, respectively (May et al., 1990). Other studies have not seen such definitive changes; describing a mild, but nonsignificant change with age in PD patients from 0.47 to 0.40 ml/min (Silverberg et al., 2001). Noninvasive techniques generally provide higher overall estimates for CSF secretion, but there have not been sufficient studies looking at the oldest age groups (e.g., 75+). However, using MRI, secretion rates of 0.68 ml/min and 0.69 ml/min in a group of young (average 30 years) and older (average 69 years) subjects was measured (Gideon et al., 1994). Animal studies have provided more consistent findings, with age-related decrease in CSF secretion in rats and sheep (Preston, 2001; Wilson et al., 1999). In these models, like in NPH, ion transport deficits are seen in the reduced blood to CSF transport of Na⁺ in the rat in vivo (Smith et al., 1982), reduced Na⁺, K⁺-ATPase activity and mRNA expression in the rat CP (Kvitnitskaia-Ryzhova and Shkapenko, 1992; Masseguin et al., 2005), and in the sheep, reduced Na⁺ uptake and efflux (Chen et al., 2005b), and Cl⁻ efflux (Preston, 1999). The aging sheep CP is also less sensitive to inhibitors of CSF secretion, such as ouabain and acetazolamide (Chen et al., 2005b), and to an upregulator of K⁺/Cl⁻ transport, NEM (Chen et al., 2004). In addition, studies have shown reduced expression of carbonic anhydrase II (providing the HCO₃⁻ for Cl⁻ exchange) and aquaporin 1, consistent with reduced water flux across the apical CP membrane (Masseguin et al., 2005).
a. Vasopressin. Potential underlying mechanisms for such changes are not clearly defined, but will reside both in the general age-related changes to the CNS and vasculature impacting on brain mass, central sinus pressure, and CP perfusion, as well as to CP-specific decrements. Arginine vasopressin (AVP) is likely to play a part in the CP-specific changes. With increasing age (from 25 to 75 years old), AVP levels in plasma rise more than six-fold in humans (Frolkis et al., 1982) and this was confirmed in later studies (Johnson et al., 1994; Lucassen et al., 1997). CSF levels are also seen to rise in the aging rat, although not in water deprivation state (Frolkis et al., 1999), and there is an increase in the number of AVP-positive fibers penetrating the third ventricle and forming “axoventricular” contacts (Frolkis et al., 1999). AVP has inhibitory effects both on CP perfusion and CSF secretion (Chodobski et al., 1998b; Faraci et al., 1988) and induces “dark” epithelial CP cells in young tissue and reduced Cl⁻ flux via the V₁ receptor (Johanson et al., 1999a). Similar morphological changes are seen in aged mouse CP, where dark cell numbers are increased (Sturrock, 1988), and in rat, Cl⁻ efflux is reduced (Preston, 1999). Interestingly, CP dark cells are also evident in animal models of hydrocephalus (Shuman and Bryan, 1991) and it has been suggested that the presence of dark cells is indicative of CSF “resorption” in a situation in which there is excess ventricular CSF (Weaver et al., 2004).

b. Fibroblast Growth Factor 2. Age-related changes in FGF2 levels in CP or CSF have not been systematically assessed. What little data there is suggests no changes to either serum or CSF levels with healthy aging in humans (Johansson et al., 2003), although CSF elevations are seen in the neurological disorder amyotrophic lateral sclerosis (Johansson et al., 2003) and in brain parenchyma in AD (Stopa et al., 1990). Data for normal brain levels vary depending on cell type and whether protein immunoreactivity or mRNA is assessed (Belluardo et al., 2004; Cintra et al., 1994; Lolova, 1991), but overall there is a trend for an age-related increase in baseline FGF2 mRNA in aging rat striatum and cerebral cortex (Belluardo et al., 2004). Nicotine treatment produces significant upregulation of FGF2 mRNA in the cerebral cortex, hippocampus, and substantia nigra of both adult and aged rats (12 and 24 months compared to 3 months) via nicotinic receptors and is neuroprotective (Belluardo et al., 2004). Although elevated FGF2 may be beneficial for neuronal survival following trauma and stimulate neurogenesis (Jin et al., 2003), the evidence that it can induce hydrocephalus via increased resistance to CSF drainage (Johanson et al., 1999b) suggests that high brain levels may contribute to dysfunction of CSF dynamics in aging and disease. FGF2 also induces CP epithelial dark cells and reduces CSF secretion rate, possibly via interaction with AVP (Szmydynger-Chodobska et al., 2002), and so may exacerbate the AVP effect on CP.
c. Cytokines. Marked elevation in CSF cytokines interleukin-1β, tumor necrosis factor-α (TNF-α), and TGF-β are seen in various neurodegenerative states with underlying inflammation (Flood et al., 2001; Sjogren et al., 2004; Tarkowski et al., 2003a) and a particularly marked elevation in TNF-α is seen in NPH with accompanying neuronal degeneration and ependymal disruption (Tarkowski et al., 2003b). However, only TGF-β levels seem to show any correlation with age, increasing in CSF between 50 and 83 years (Sjogren et al., 2004). Production is regulated by other cytokines, including TNF-α, and TGF-β acts as an anti-inflammatory cytokine, inhibiting, by negative feedback, production of proinflammatory cytokines, such as TNF-α. The pattern of cytokines is fundamentally different in aging CSF compared to NPH and probably indicates the relative preservation of brain parenchyma in healthy aging.

B. CP Senescence: An AD Connection?

1. Morphological and Biochemical Changes

Whether reduced CSF secretion is a cause of the altered volume transmission seen with aging or a consequence of it, there is abundant additional evidence for global dysfunction of the CP. Pronounced morphological changes occur, including multiple intracellular accumulations of lipofuscin (Serot et al., 2000; Wen et al., 1999), psammoma bodies (Jovanovic et al., 2004), amyloid Biondi bodies (Eriksson and Westermark, 1990; Miklossy et al., 1998), and calcification (Modic et al., 1980). The epithelial cells become flattened and the microvilli are shortened (Serot et al., 2003) reducing the apical surface area, and many of these changes are exaggerated in AD. Extracellular fibrosis of the stroma and thickening of the basement membrane (Serot et al., 2000, 2001; Shuangshoti and Netsky, 1970) would additionally act to impede fluid movement across the tissue in either direction. Generalized oxidative damage to CP nuclear DNA is seen (Nakae et al., 2000), with increased mitochondrial dysfunction in aged humans (Cottrell et al., 2001), decline in activity of enzymes of anaerobic and oxidative respiration (Ferrante and Amenta, 1987), and elevation in CSF lactate (Yesavage et al., 1982), all of which point to declines in energy transduction, essential for maintenance of normal CP function.

2. Protein and Peptide Synthesis

TTR is a major protein synthesized by the CP and secreted preferentially into CSF. Published data on TTR changes in CSF with age appear conflicting, and elevated (Kleine et al., 1993; Serot et al., 1997), stable
(Garton et al., 1991; Kunicki et al., 1998; Vatassery et al., 1991), and declining levels have been reported (Zheng et al., 2001). What is consistent is the finding of reduced CSF TTR in patients with AD (Riisoen, 1988; Serot et al., 1997). In animal models, we have described reduced TTR in aged sheep CSF (Chen et al., 2003). In these animals, there is no change in TTR mRNA expression in the CP (Chen et al., 2005a) or changes in plasma TTR levels, but there was a reduction in de novo protein synthesis by the aging CP and a reduction in newly synthesized TTR monomers both in the CP and in the newly secreted CSF in old sheep (Chen et al., 2005a). TTR has several roles within CSF, including being a chaperone protein for T4 (previously described) and retinoic acid (see Zheng et al., 2001), and importantly, prevents β-amyloid peptide (Aβ) fibril formation and hence neurotoxicity (Schwarzman et al., 2004). The importance of T4 in maintaining mature neuronal metabolism and of amyloid in the pathogenesis of AD has led to the suggestion that lack of sufficient TTR contributes directly to age-related cognitive decline (Rubenstein, 1998). Evidence to suggest that the CP-CSF axis plays a role in Aβ homeostasis with potential for modulating the onset and course of AD includes nicotine- and estrogen-induced TTR increase in brain and CP (Li et al., 2000; Tang et al., 2004) and may help explain the protective effect of cigarette smoking and estrogen in dementia (Graves et al., 1991; Tang et al., 1996). In a transgenic mouse carrying a mutant form of human amyloid precursor protein, the mechanism of protection appears to lie in upregulation of TTR given that administration of antibody against TTR results in neuronal loss and apoptosis (Stein et al., 2004).

The CP also expresses mRNA for other proteins involved in amyloid handling or with a protective role, including IGF2, which is neuroprotective in vitro (Zheng et al., 2000), IGFBP2, which affects the action of IGF2 (Walter et al., 1999), gelsolin, which inhibits Aβ fibrillogenesis and prevents neurotoxicity (Matsumoto et al., 2003; Qiao et al., 2005; Ray et al., 2000), and the apolipoprotein J receptor, LRP2 (Kounnas et al., 1994), which mediates cellular clearance of Aβ₁₋₄₀ (Hammad et al., 1997). We know little about the effect of late life on production of these compounds, although it is known that production of IGF is partially dependent upon the presence of GH (Cohen et al., 1992), which falls in plasma in later life (Arnold et al., 1999), with reduced density of GH binding to CP (Lai et al., 1993). Because IGF2 may also have a role in promoting choroidal epithelial cell growth (Nilsson et al., 1996), any reduction in activity or content in the CP could have significant consequences for tissue repair and cell turnover. More generally, the global decline in de novo protein synthesis by aged sheep CP and the subsequent reduction in new proteins secreted into CSF (Chen et al., 2005a) could compromise all of these maintenance and protective mechanisms.
C. CSF Turnover and Clearance

For the aging CSF system, like NPH, any one of the gross changes described (increased intracranial CSF volume, increased $R_{\text{out}}$, or decreased CSF secretion) would be sufficient to increase the turnover time of the CSF leading to stagnation of the fluid and impairment of volume transmission. In aging all these factors are likely to be present, and the rate at which CSF is replaced falls from around 4 times each day to 1–2 times a day in humans (Rubenstein, 1998; Silverberg et al., 2003) and from 12 to 3 times a day in rats (Preston, 2001). As a consequence, the clearance from CSF of a range of compounds slows with increasing age. For example, after lumbar injection of radioiodinated human serum albumin (RIHSA), clearance from aged human CNS is slow and most RIHSA can be detected in the brain 1–2 days later, in contrast to younger subjects when most RIHSA had been cleared (Henriksson and Voight, 1976). Compounds smaller than albumin also show reduced clearance. The rate of removal of ICV-injected radiolabeled $\alpha\beta$ fell by 90% between 3 and 30 months of age, and at the same time, brain accumulation of the labeled $\alpha\beta$ increased from 7% to 49% (Preston, 2001). Similarly, the old senescence-accelerated mouse, SAMP8, shows slower efflux of radiolabeled $\alpha\beta_{1-42}$ from brain after ICV injection compared to young SAMP8 or control mice (Banks et al., 2003).

Many other proteins, particularly of large molecular size, have increased CSF/plasma ratios with age. For example, albumin in humans and sheep and IgG in humans (Blennow et al., 1993; Chen et al., 2003; Garton et al., 1991; Reiber, 2001). Brain-derived proteins have a more varied pattern (Reiber, 2001) presumably because their removal depends on brain ISF flow and the effect of the local BBB as well as CSF drainage. Because studies on both sheep and rats show no significant change in BCSFB permeability for large compounds (Chen et al., 2005b; Preston, 2001), the elevation of CSF proteins is consistent with reduced CSF turnover (Reiber, 2001) rather than an increased BCSFB or BBB permeability.

A further consequence of stagnation in CSF turnover is impaired delivery of compounds by CSF to brain. Direct studies are lacking; however, studies of FGF2 administration into lateral ventricles of young and old mice suggest that this may be a factor. FGF2 administration results in increased neurogenesis in the SVZ and hippocampal dentate gyrus in both young and old mice, demonstrating the potential for neuronal replacement even into old age (Jin et al., 2003). However, the effects in old mice were mostly seen in the ipsilateral SVZ after unilateral ICV injection, while in young mice, similar increases in neurogenesis were observed in both hemispheres after unilateral injection (Jin et al., 2003). Transiently higher levels of growth factor in the ipsilateral ventricle immediately after injection is put forward as an explanation by the authors, and this is consistent with alterations in CSF dynamics.
and stagnation of ventricular pools in the old rats using CSF as a vehicle for CNS distribution. It is notable that the old SVZ is capable of continuing to act as a nursery for neurogenesis after FGF2 administration; that this is limited \textit{in vivo} may relate to availability of CSF-delivered growth factors, as well as neuronal function in late life.

The consequences of restoring CSF turnover on cognitive function in AD have been investigated (Silverberg \textit{et al.}, 2003). A ventricular shunt was established to increase CSF drainage and this was successful in stabilizing the cognitive deficit so that at 12 months after shunt, no change in Mattis dementia rating scale was seen. In the nonshunted group, cognitive decline followed its expected course (Silverberg \textit{et al.}, 2003).

From human and animal studies of healthy later life and disease, it is clear that there is senescence-related dysfunction in multiple aspects of the CP-CSF axis. CP function, CSF secretion, and CSF clearance are all negatively affected, resulting in reduced volume transmission with detrimental effects both on the removal of compounds from the CSF/CNS and on the capacity to act as a vehicle for distribution of essential compounds throughout the CNS. The consequences for global CNS function need now to be systematically addressed.

\section*{VI. Perspectives}

The experimental and clinical data previously discussed support the importance of the CP-CSF system in CNS development, homeostasis and repair, and aging. The CP is a highly specialized tissue, strategically positioned within the ventricular cavities to provide the CNS with a variety of biologically active factors that are essential for normal brain function. Shown in Table I, the CP synthesizes a number of neurotrophic and angiogenic factors, chemorepellents, and carrier proteins, as well as cell-associated and secreted enzymes, and enzyme inhibitors. The CP is also equipped with many transport systems that not only control the entry of nutrients and other essential substances from the periphery into the brain, but that also play important roles in the clearance of toxins and brain metabolites.

The diffusible growth factors and chemorepellents secreted by the CP appear to be critically involved in neurogenesis and axonal guidance during the development of the CNS. Therefore, a better understanding of the role that the CP-CSF system plays in brain development is likely to benefit in new treatments of congenital CNS disorders. Various functions of immature choroidal tissue subside in the adult CNS; however, after brain injury, the CP resumes its ability to promote neuronal survival and to restore the brain microenvironment. A growing body of evidence suggests that the CP can act as a nursery for neuronal and astrocytic progenitor cells. The demonstrated
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<th>Peptide/Protein</th>
<th>Putative Functions</th>
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<tr>
<td><strong>Growth factors</strong></td>
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<td>Bone morphogenetic proteins 4–7 (BMP4–7)</td>
<td>Autocrine and/or juxtacrine/paracrine regulation of CP development. May be involved in postischemic neuronal repair processes in the hippocampus</td>
<td>Charytoniuk et al., 2000; Furuta et al., 1997; Hébert et al., 2002</td>
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<tr>
<td>Fibroblast growth factor 2 (FGF2)</td>
<td>Autocrine and/or juxtacrine/paracrine regulation of CP development. May play a role in the development of the cerebral cortex. In adult animals, it promotes the differentiation of progenitor cells in the subventricular zone (SVZ) into neurons. Inhibits cerebrospinal fluid (CSF) formation. Overproduction of FGF2 may cause fibrosis of the arachnoid villi and ventricular enlargement</td>
<td>Gonzalez et al., 1995; Jin et al., 2003; Johanson et al., 1999b; Kuhn et al., 1997; Ortega et al., 1998; Raballo et al., 2000; Reid and Ferretti, 2003</td>
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<td>Growth/differentiation factor 15 or macrophage-inhibiting cytokine 1 (GDF-15/MIC-1)</td>
<td>Survival-promoting factor for dopaminergic neurons</td>
<td>Schober et al., 2001; Strelau et al., 2000</td>
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<td>Insulin-like growth factor 2 (IGF2)</td>
<td>Early expression of IGF2 in the choroidal stroma may promote the differentiation of choroidal epithelial cells, whereas epithelium-derived IGF2 may be involved in the development of other parts of the brain. May play a role in repair processes after brain injury</td>
<td>Bondy et al., 1992; Cavallaro et al., 1993; Logan et al., 1994; Walter et al., 1999</td>
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<tr>
<td>Heparin-binding epidermal growth factor-like growth factor (HB-EGF)</td>
<td>Promotes neurogenesis in the SVZ</td>
<td>Jin et al., 2003; Mishima et al., 1996</td>
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<tr>
<td>Transforming growth factor-α (TGF-α)</td>
<td>Neuroprotection in ischemic brain injury</td>
<td>Diaz-Ruiz et al., 1993; Justicia et al., 2001</td>
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Transforming growth factor-β (TGF-β; isoforms 1, 2, and 3; TGF-β3 is expressed in embryonic CP, whereas TGF-β1–3 are expressed in adult CP)  
May be involved in normal pattern formation and the specification of neural progenitor populations in the dorsal neural tube. May play a role in the induction and survival of dopaminergic neurons in the midbrain. Exerts neuroprotective effect on the CA1 hippocampal region. Postinjury overproduction may result in hydrocephalus  
Chesnutt et al., 2004; Farkas et al., 2003; Flood et al., 2001; Henrich-Noack et al., 1996; Knuccey et al., 1996; Pelton et al., 1991; Tada et al., 1994

Vascular endothelial growth factor (VEGF)  
Antagonizes the actions of chemorepellents. Promotes neurogenesis in the SVZ and dentate gyrus. Provides neuroprotection in ischemic brain injury  
Bagnard et al., 2001; Chodobski et al., 2003; Schänzer et al., 2004; Sun et al., 2003

**Chemorepellents**

Semaphorin 3F (SEMA3F)  
During embryonic development, repels the axons of the epithalamic and hippocampal neurons  
Tamada and Murakami, 2004

Slit proteins (SLIT2 and SLIT3)  
During embryonic development, SLIT2 repels the precursors of olfactory interneurons  
Hu, 1999; Nguyen-Ba-Charvet et al., 2004; Tamada and Murakami, 2004

**Cytokines and chemokines**

Interleukin-1β (IL-1β)  
Host-defense response to infection  
Quan et al., 1998, 1999

Tumor necrosis factor-α (TNF-α)  
Host-defense response to infection  
Quan et al., 1999; Tarlow et al., 1993

Cytokine-induced neutrophil chemoattractants 1 and 2α (CINC1 and CINC2α)  
Promote neutrophil migration across the blood-CSF barrier (BCSFB)  
J. Szmydynger-Chodobska and A. Chodobski (unpublished observations)

**Neuropeptides**

Adrenomedullin (ADM)  
Controls CSF formation and/or other CP functions by up-regulating choroidal 3',5'-cyclic monophosphate synthesis. Modulates the permeability of the blood-brain barrier  
Kis et al., 2001, 2003; Kobayashi et al., 2001; Takahashi et al., 1997

Arginine vasopressin (AVP)  
Inhibits CSF formation  
Chodobski et al., 1997, 1998b; Johanson et al., 1999a

Endothelin 1 (ET1)  
May play a role in the development of cerebral vasospasm after subarachnoid hemorrhage  
Takahashi et al., 1998; Zimmermann and Seifert, 1998

(Continued)
Table I  Continued

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<th>Peptide/Protein</th>
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<td><strong>Carrier proteins</strong></td>
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<tr>
<td>Apolipoprotein J (apoJ)/clusterin (CLU)</td>
<td>Binds β-amyloid peptide (Aβ) and prevents its aggregation and polymerization</td>
<td>Aronow et al., 1993; Matsubara et al., 1996</td>
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<td>Gelsolin (GSN)</td>
<td>Inhibits the fibrillization of Aβ and promotes the disaggregation of preformed β-amyloid fibrils</td>
<td>Matsumoto et al., 2003; Ray et al., 2000</td>
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<tr>
<td>Transthyretin (TTR)/prealbumin</td>
<td>May play a role in the transport of thyroxine (T₄) across the BCSFB. Inhibits aggregation of Aβ</td>
<td>Cavallaro et al., 1993; Dickson and Schreiber, 1986; Schreiber et al., 1990; Schwarzman et al., 2004; Southwell et al., 1993</td>
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<td><strong>Enzymes</strong></td>
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<td>Alcohol dehydrogenases (ADH1 and ADH4 or ADH3—conflicting data)</td>
<td>Convert retinol to retinal, the precursor of retinoic acid (RA). RA produced by the fourth ventricle CP may play a role in the development of the cerebellum</td>
<td>Galter et al., 2003; Marinez et al., 2001; Yamamoto et al., 1996</td>
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<tr>
<td>Matrix metalloproteinases 2 and 9 (MMP2 and MMP9)</td>
<td>Facilitate leukocyte migration across the BCSFB</td>
<td>Strazielle et al., 2003</td>
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<tr>
<td>Prostaglandin H synthase and prostaglandin D synthase/β-trace protein (PGHS and PGDS)</td>
<td>By sequential actions, produce prostaglandin D₂ (PGD₂) from arachidonic acid. May play a role in controlling CP function because the receptors for PGD₂ are expressed in the CP</td>
<td>Hoffmann et al., 1996; Thrikawala et al., 1998; Wright et al., 1999</td>
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<tr>
<td>Retinaldehyde dehydrogenase 2 (RALDH2/ALDH1A2)</td>
<td>Converts retinal to RA. RA produced by the fourth ventricle CP may play a role in the development of the cerebellum</td>
<td>Yamamoto et al., 1996, 1998</td>
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<td><strong>Enzyme inhibitors</strong></td>
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<tr>
<td>Cystatin C (CST3)</td>
<td>Neuroprotection in focal cerebral ischemia</td>
<td>Olsson et al., 2004; Tu et al., 1990, 1992</td>
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<td>Tissue inhibitor of metalloproteinase 3 (TIMP3)</td>
<td>Inhibits CP-derived MMP2 and MMP9. Blocks the binding of VEGF to its type II receptor in the CP</td>
<td>Butler et al., 1999; Pagenstecher et al., 1998; Qi et al., 2003</td>
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neuroprotective capabilities of choroidal transplants open new and exciting avenues of research that may lead to designing novel therapies for ischemic stroke, neurotrauma, and neurodegenerative diseases. Furthermore, understanding the mechanisms that lead to the senescence-related dysfunction of the CP-CSF axis may help find the treatments needed to reverse the negative effects of aging that lead to global CNS failure. Accordingly, the challenge for the coming decades is to learn how to bring into clinical practice the potential of the CP to control brain homeostasis and assist in the repair processes of the CNS. It is hoped that this analysis of the various aspects of CP function will attract the broader attention of researchers outside the CP field.

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References


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