Pharmaceutical Research, Vol. 22, No. 7, July 2005 (© 2005)
DOI: 10.1007/s11095-005-6039-0

Expert Review

Enhanced Prospects for Drug Delivery and Brain Targeting by the Choroid Plexus–CSF Route

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Received February 18, 2005; accepted April 12, 2005

Abstract. The choroid plexus (CP), i.e., the blood–cerebrospinal fluid barrier (BCSFB) interface, is an epithelial boundary exploitable for drug delivery to brain. Agents transported from blood to lateral ventricles are convected by CSF volume transmission (bulk flow) to many periventricular targets. These include the caudate, hippocampus, specialized circumventricular organs, hypothalamus, and the downstream pia–glia and arachnoid membranes. The CSF circulatory system normally provides micronutrients, neurotrophins, hormones, neuropeptides, and growth factors extensively to neuronal networks. Therefore, drugs directed to CSF can modulate a variety of endocrine, immunologic, and behavioral phenomema; and can help to restore brain interstitial and cellular homeostasis disrupted by disease and trauma. This review integrates information from animal models that demonstrates marked physiologic effects of substances introduced into the ventricular system. It also recapitulates how pharmacologic agents administered into the CSF system prevent disease or enhance the brain's ability to recover from chemical and physical insults. In regard to drug distribution in the CNS, the BCSFB interaction with the blood–brain barrier is discussed. With a view toward translational CSF pharmacotherapy, there are several promising innovations in progress: bone marrow cell infusions, CP encapsulation and transplants, neural stem cell augmentation, phage display of peptide ligands for CP epithelium, CSF gene transfer, regulation of leukocyte and cytokine trafficking at the BCSFB, and the purification of neurotoxic CSF in degenerative states. The progressively increasing pharmacological significance of the CP–CSF nexus is analyzed in light of treating AIDS, multiple sclerosis, stroke, hydrocephalus, and Alzheimer's disease.

KEY WORDS: blood–CSF barrier; brain drug delivery; cerebrospinal fluid; choroid plexus; CSF bulk flow; CSF pharmacokinetics; intracerebroventricular; volume transmission.

DISTINCTIVE FEATURES OF THE BLOOD–CSF BARRIER

Choroid Plexus *vs.* other CNS Transport Interfaces

Choroid plexus (CP) is a secretory epithelial tissue suspended at multiple loci in the cerebroventricular system. In addition to manufacturing the CSF, it performs a diversity of homeostatic functions to stabilize the interstitial environment of neurons. Kidney, liver, and immune-type functions have been ascribed to CP. This gives it pathological and therapeutic significance for a multitude of reasons (1). CP has a prominent role in fetal CNS development, especially having an impact on the periventricular neurogenic zones. In late stages of life, when the brain is challenged with degenerative diseases, the turnover rate of CSF and its constituents become a critical factor in neural viability. In health, the CP–CSF nexus furnishes micronutrients, growth factors, and neurotrophins to neuronal networks. This is the rationale for developing pharmacological agents to distribute along similar CSF pathways to targets in the brain.

Both the CP and arachnoid membrane comprise the blood–CSF barrier (BCSFB) (1–3). Only the former, however, has received serious attention for drug transport and metabolism. Drug permeation of epithelia depends on a number of anatomical and physiological factors (Table I). Interpreting CNS pharmacokinetic data is complicated by molecular fluxes across several transport interfaces and the compartments that they demarcate (4). Figure 1 depicts compartmental relationships. Transport sites display a broad spectrum of physical "barrier" impermeabilities, fluid turnover rates, and facilitated transport mechanisms. Blood-borne agents penetrate the CNS transport interfaces, or "barriers," mainly via the cerebral microvessels and the

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Table I. Histological and Functional Features of Mesenchymal Support Structures that Form Epithelial Borders with the CSF

Epithelial type	CSF region	Junctions	Layer(s)	Functions[a]	References
Choroid plexus	LV, 3V, 4V[b]	Tight	Single	Forms the CSF and secretes proteins, ions, and micronutrients	(21,24,30,38,68)
Ependyma	LV, 3V, 4V	Leaky[c]	Single	Allows the CSF "sink action" on the brain due to high permeability	(11,56,57,59,61,62)
CVOs	3V, 4V	Tight	Single	Integrates hormonal and neural activity to effect fluid homeostasis	(2,38,75,76,111)
Pia–glia	SAS[d]	Leaky	Multiple	Protects brain by filtering and buffering actions on SAS CSF	(3,148,149)
Arachnoid	SAS	Tight	Multiple	Secretes neurotrophic peptides and reabsorbs CSF in the villi	(1–3,34,40,127,129)

[a] Virtually all epithelia in the CSF system can metabolize drugs.
[b] LV, 3V, and 4V refer to the lateral, third, and fourth ventricles, respectively.
[c] In the third ventricle area, there are tight junctions between some ependymal cells.
[d] SAS = subarachnoid space; CVOs = circumventricular organs.

choroid plexuses of the lateral, third, and fourth ventricles. The choroidal epithelium, however, has structural and functional properties that distinguish it from the cerebral endothelium of the BBB. The unique characteristics of the CP interface (Fig. 2) prompt consideration of drug delivery (5) to the CNS by way of CSF (6).

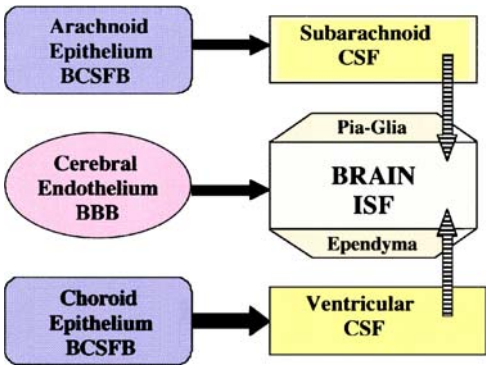

Fig. 1. Schema depicting *inward* solute transfer routes to the central nervous system: Three major transport interfaces work in parallel to provide plasma solutes for the neural networks. Brain capillary endothelial cells, comprising the blood–brain barrier (BBB), directly provide a variety of nutrients and trophic molecules to the brain interstitial fluid (ISF). The epithelial cells of the arachnoid membrane and the choroid plexuses constitute two separate blood–CSF barriers (BCSFBs). They furnish a different spectrum of substances to the brain indirectly by way of the intermediary CSF, which bathes the cerebral exterior and interior surfaces. Of the three major blood–CNS interfaces depicted on the left side of the diagram, the secretory capacity for solute and water transport at the choroid plexus BCSFB is the greatest. Plasma-carried solutes move across the BBB and BCSFB mainly by active transport and facilitated mechanisms (solid black arrows) that regulate inward transfer across tight-junction sealed membranes. On the other hand, upon accessing the CSF, substances can readily penetrate the brain exterior and interior, respectively, across the pia–glial and ependymal linings that have permeable gap junctions. Solutes thus move easily into brain from CSF via the passive processes (stippled gray arrows) of simple diffusion or convective bulk flow (volume transmission) dependent upon hydrostatic pressure gradients. Bidirectional inward and outward transport occurs at all interfaces depicted; however, this review emphasizes transport of substances into the brain.

Choroidal Blood Flow and Interstitial Fluid Dynamics

As a transport "crossing," the CP is often viewed as conducting far less extensive molecular exchange than the BBB. This argument is based on earlier estimates of a smaller surface area of CP relative to the BBB (7,8). A host of factors promoting transport, however, need to be weighed. Choroidal

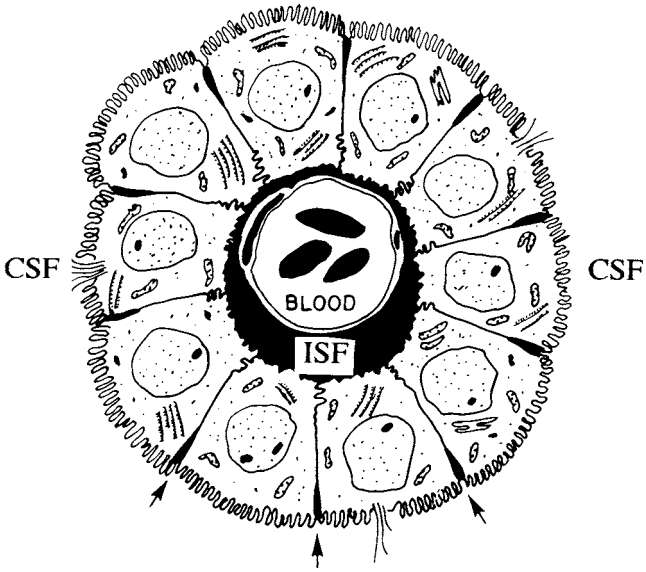

Fig. 2. Cross-section of a typical choroid plexus villus reconstructed from electron micrographs: The vascular core of each villus is surrounded by a single-layer ring of epithelial cells (ca. 10-μm cubes). Arrows point to tight junctions at the apical (CSF-facing) pole of adjoining epithelium. Intervening between the blood compartment and the epithelial parenchyma is an interstitial fluid (ISF) compartment comprising about 15% of the choroidal tissue volume. CSF is generated as water and ions stream out of the permeable capillaries into the ISF, and then move transcellularly (across) and paracellularly (between) the epithelial cells into ventricular CSF (38,39). The potentially rate-limiting step in the CSF uptake of many water-soluble drugs would be active transport across the basal membrane at the ISF interface. Anatomic–physiological relationships are similar for the villi of the lateral, third, and fourth ventricles. Unless otherwise stated in the text, most of the CP data is for the lateral ventricle that houses most of the choroidal tissue mass.

vascular perfusion is five to ten times that of the mean cerebral blood flow (9,10). This brisk choroidal blood inflow provides plentiful substrate and water for secretion. Plexus capillaries containing gap junctions are markedly more permeable than brain microvessels having tight junctions. Consequently, in the CP vascular wall the initial transport step in the blood-to-CSF distribution of materials is not rate limiting as is the case with the BBB microvessels. Moreover, the choroidal interstitium adjoining the vasculature normally does not appreciably impede diffusing ions and molecules. Therefore the leaky choroidal capillaries in series with the low-resistance interstitial zone allows relatively free diffusion of solutes from plasma up to the basolateral membrane of the epithelium.

Choroid Epithelial Surface Area and Transport

Extensive basolateral infoldings provide a considerable surface for transport. In addition, the lush apical membrane microvilli (Fig. 3) impart a massive surface area for molecular fluxes. It is becoming more appreciated that the total area for transport by the four CPs is the same order of magnitude as the entire BBB (11). Substantial secretion and reabsorption are energized by a profusion of mitochondria. Ultrastructural profiles of organelles reveal cellular machinery specialized for a high-level transport of ions driving the fluid movement (12). Interestingly, about 85% of Na and Cl flux into rat CNS occurs preponderantly via the CP–CSF (13) compared to a mere 15% at the BBB. Besides, certain vitamins and hormones reach brain by way of carrier transport in CP rather than across cerebral capillaries. Altogether, the blood–CSF interface in the four ventricles regulates substantial fluxes.

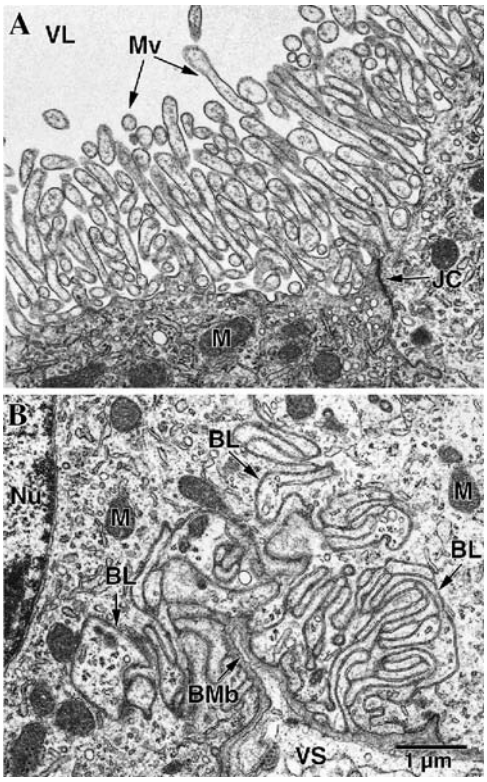

Fig. 3. Surface area configurations in the apical and basal membranes of choroid plexus epithelium in the lateral ventricle of the adult rat. The ultrastructural morphology is typical of the choroid plexus of several mammalian species, including human. Top (A): The apical membrane has a lush microvilli (Mv) system that allows substantial active transport of ions and molecules between CSF and the epithelial cytoplasm. M, mitochondrion; JC, junctional complex (i.e., tight junction or *zonulae occludentes*) between adjacent choroid cells impedes the diffusion of water-soluble substances between the paracellular space and the ventricular CSF. Bottom (B): The basal labyrinth (BL) at the bottom of the cell, near the interstitial space, has extensive membrane infoldings that impart a large surface area for the active transport of substances between interstitial fluid and choroidal cytoplasm. Nu, nucleus; Bmb, basement membrane; VS, vascular space.

Epithelial Tight Junctions and Paracellular Diffusion

Yet another distinction between CP and brain microvessels is the nature of the tight junctions at the respective blood–CSF and blood–brain barriers. The tight junctions, which contain the protein occludin (14), are also known as *zonulae occludentes*. They are "spot welds" at the apical zone of neighboring epithelial cells. These tight junctions partially occlude or block the passage of water-soluble agents (depending on molecular size) between the parenchymal cells of the barriers, i.e., as the particular solute diffuses from blood to CSF or brain. However, less restriction to the diffusion of polar substances is offered at the blood–CSF interface than the BBB (15). This is attributable to the more permeable *zonulae occludentes* between choroidal epithelial cells compared with the counterpart junctions joining the cerebral endothelia (14). Consequently, there is paracellular diffusion of small hydrophilic solutes through CP tight junctions and onward into CSF. For example, intravascularly administered mannitol (M_W = 182) and inulin polysaccharide ($M_W \sim 5{,}500$) penetrate into CSF (16,17) by diffusing between choroid epithelial cells rather than through them (18,19).

Tight junctions in CP demonstrate fluidity by undergoing reversible changes in the augmented permeability induced by hyperosmoticity (20,21). Given the malleable nature of *zonulae occludentes* and their alteration in disease, it should be feasible to design therapeutic regimens (22) or new polar agents such as stavudine (15) to promote drug access to CSF across choroidal tight junctions.

Fluid-Producing Capacity of Choroid Plexus *vs.* BBB

Fluids formed at the barrier systems affect drug distribution in the CNS. The BCSFB and BBB differ prominently in fluid-producing capacity. By transporting great amounts of Na, Cl, and water, the CP elaborates a copious volume of CSF. The CP epithelium, with its abundant carbonic anhydrase activity, generates CSF at 0.4 ml/min/g. On the other hand, BBB fluid production rate is markedly less. This pronounced difference is reflected in the ability of acetazolamide, a carbonic anhydrase inhibitor, to curtail ^{22}Na transport from blood to CSF by 30%, but not to alter ^{22}Na transport across the BBB (23). In light of the preponderant Na transport at the BCSFB (13), it is evident that the coupled ion–water movement accesses the CNS mainly via the CPs. The sustained, extensive translocation of water from blood to ventricular CSF entrains polar molecules. Consequently, the immense movement of water across CP epithelium drives the CSF convection of transported nutrients and drugs throughout the brain.

Differential Expression of Transporters

Due to distinct epithelial *vs.* endothelial phenotypes, it is pharmacologically useful to examine transporter expression at the BCSFB *vs.* BBB. Two significant examples are the Na ascorbate cotransporter and the truncated receptor for prolactin. Both are expressed by CP epithelial cells, but apparently not by brain microvessel endothelium (24–26). Substantial active transport of ascorbate has been demonstrated at the BCSFB (27), but not at the BBB. Vitamin C supply to the CNS evidently involves conveyance of large amounts of ascorbate to brain parenchyma (28) via CSF routes (27). In the case of prolactin, this blood-borne hormone reaches the CSF by a carrier in CP (26,29) evidently

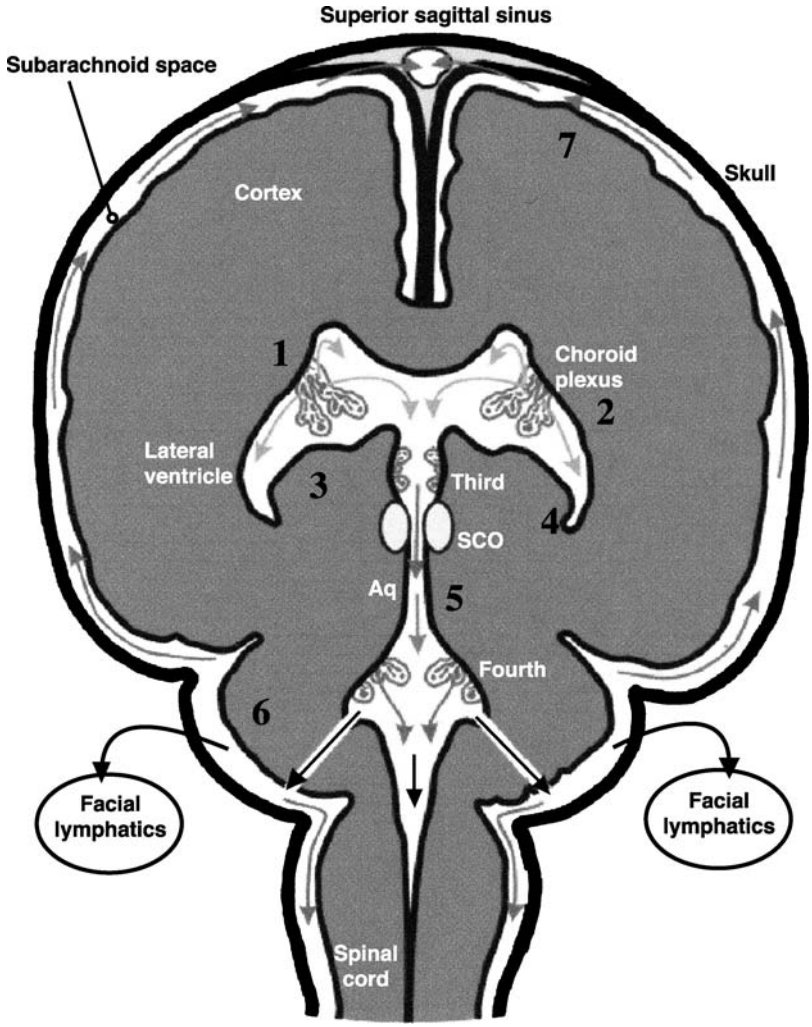

Fig. 4. CSF flow pathways in the adult: Nearly all major brain structures interface with the CSF system. This coronal section of the posterior CNS captures all the ventricles: lateral, third, and fourth. CSF originates from the choroid plexuses of the lateral and third ventricles, and percolates downward through the narrow cerebral aqueduct (Aq). The bottom of the Aq empties into the fourth ventricle, to the roof of which is attached more choroidal tissue. CSF flows out of the fourth ventricle through foramina into the cisterna magna and other nearby large basal cisterns. From the cisterns, the CSF is convected posteriorly and downward around the spinal cord (subarachnoid space) as well as upward over the convexities of the cerebral hemispheres. At more distal sites in the subarachnoid system, the CSF flows outward through the arachnoid villi into venous blood of the superior sagittal sinus. Arrows depict the general patterns of CSF flow, from the interior of the brain to various exterior loci in the spinal cord and hemispheres. SCO, subcommissural organ. Some CSF drains directly into lymphatic glands. 1, Caudate; 2, centrum ovale (white matter); 3, thalamus; 4, hippocampus; 5, periaqueductal gray; 6, cerebellum; 7, cerebral motor cortex. (This is a modified diagram by Miyan *et al.* (59), used with permission.) Anatomical relationships among the pia mater, arachnoid, subarachnoid space CSF, and egress drainage sites in the dura mater, are delineated in Fig. 5.

not present in cerebral microvessels. Proteins such as transthyretin (30) uniquely expressed in the CNS by CP are relevant to the therapy of CNS disorders (31,32).

Overall, the CP features an array of characteristics exploitable for enhancing delivery of drugs or natural substances to specific regions of brain. Distinctive molecular expression patterns in the choroidal epithelium hold promise for therapeutically manipulating the bidirectional transport of growth factors, peptides, and other organic substrates across the blood–CSF interface (6,29). Upon transport into CSF, a given drug attains a CNS distribution profile determined by numerous physiological and pharmacological factors.

DISTRIBUTIVE FUNCTIONS OF THE CP–CSF SYSTEM

CSF Flow through Large Cavities

The CSF effectively distributes native and foreign compounds. Substances presented intraventricularly, by CP secretion or pharmacological infusion, have a larger volume of distribution in CNS than those injected intrathecally above the brain and spinal cord. This is attributable to the one-way flow of CSF from the ventricles into the subarachnoid space. CSF flow routes comprise the "third circulation" (illustrated in Fig. 4). The CSF circulatory system interacts with blood, brain, and lymph. Understanding these interactions enables a greater dimensional appreciation of neuroscience physiology and brain pharmacotherapy.

This review emphasizes distribution kinetics from the viewpoint of the CP–CSF as a source of endogenous solutes and drugs for the brain. CSF streaming carries dissolved substances to regions proximate and distant from the choroidal origin. There is continuous convection of CSF, but at varying flow rates depending on pathway. The main flow involves percolation down the ventriculo-cisternal axis and then egress into the cisterns at the brain's base. Thereafter the CSF sweeps over the subarachnoid spaces encompassing the brain and cord (Fig. 4). Most distally, the CSF flows into the venous blood of the dural sinuses or the extracranial lymphatic drainage (3,33,34). Interference with major drainage routes decreases fluid turnover. This can elevate levels of drugs and metabolites in the CNS.

CSF Flow through Brain Channels

In addition to the CSF macrocirculation suffusing the interior and exterior surfaces of the brain, there is also a steady but lesser transmission of CSF along intracerebral channels. As CSF flows out of the ventricular system into the

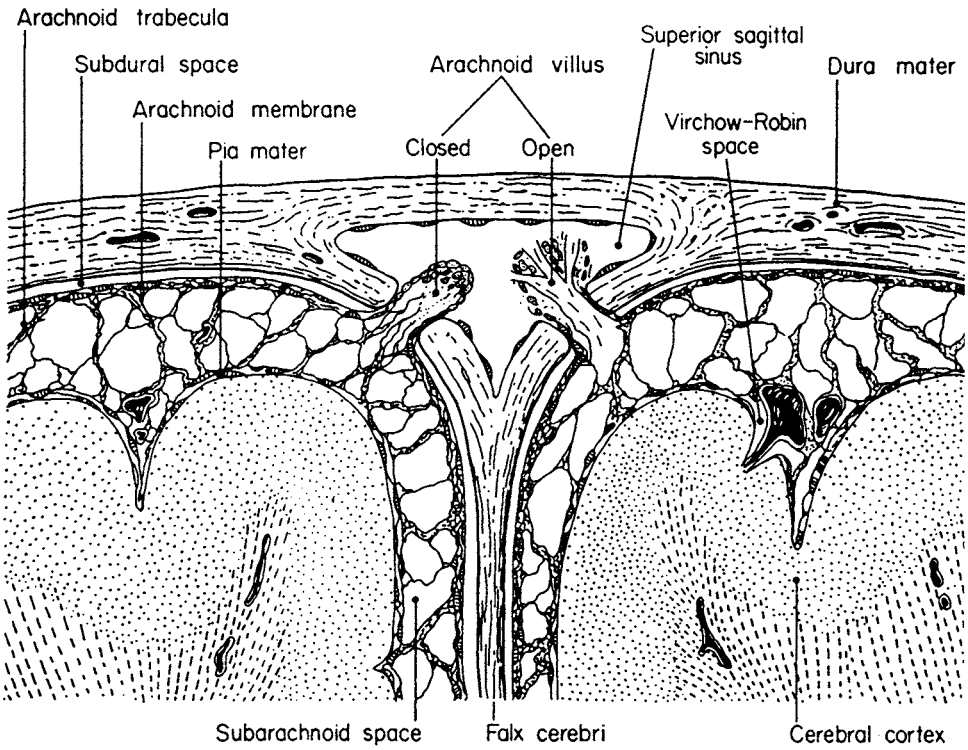

Fig. 5. Movement of CSF-borne substances through the subarachnoid spaces: Upon leaving the ventricular system, a given drug or endogenous solute can be convected throughout the spinal and cranial subarachnoid spaces. En route, an agent can either diffuse across the permeable pia mater into brain or be convected into cortical tissue via the Virchow–Robin (perivascular) spaces. Solutes remaining in the subarachnoid CSF are eventually cleared into the venous sinus across the valve-like arachnoid villi and other drainage sites in the arachnoid membrane surrounding cranial nerves. In aging and some pathological states (e.g., fibrosis in normal pressure hydrocephalus and Alzheimer's disease), there is reduced outflow of CSF into blood with consequent retention of catabolites and drugs in the CNS. Reproduce with permission by Raven Press, from the Blood–Brain Barrier in Physiology and Medicine, S.I. Rapoport, ed., 1976.

subarachnoid spaces, it can enter brain at loci where major vessels in the subarachnoid space penetrate the nervous tissue (35). Such Virchow–Robin (V–R) spaces are perivascular sleeves of CSF acting as conduits (36) for drug penetration within brain parenchyma (Fig. 5). CSF microcirculation along the V–R spaces and white matter tracts is an order of magnitude less than the ventriculo-subarachnoid macrocirculation. Still, by way of fluid spread using these various percolation routes, substances emanating from the CP (or injected into CSF) can be convected throughout the ventriculo-subrachnoid spaces to attain widespread distribution in brain (35–40).

Rapid and Pervasive Distribution of Substances from the Ventricular CSF

CSF-borne water-soluble agents such as radiolabeled sucrose ($M_W = 342$), inulin ($M_W \sim 5{,}500$), and ascorbate ($M_W = 176$) reach multiple sites in the CNS by bulk flow and diffusion along the aforementioned pathways (35–40,258). CSF is renewed at least thrice daily. Therefore the volume transmission continuously generated by CP (39) conveys substances faster than solute diffusion down concentration gradients. It is striking how rapidly and extensively substances administered intraventricularly (i.c.v.) penetrate the CNS (37,40). Within minutes, ^{14}C sucrose injected into the rat lateral ventricle flows to the third ventricle, the velum interpositum, aqueduct, fourth ventricle, and the superior medullary velum (40). CSF in these velae empties into the quadrigeminal and ambient cisterns or other recesses of the subarachnoid space. Thus i.c.v. injected sucrose, as well as inulin and ascorbate, attain widespread CNS distribution in rodent models, including substantial penetration into brain (37,258) as well as CSF-bordering regions (40).

Due to the extensive spreading of drugs in the CNS after injection into the cerebral ventricles, thousands of investigators have used the i.c.v. approach to elicit a host of central pharmacological effects. The pervasive CSF-to-brain distribution of hydrophilic test agents described above (37,40,258) fits countless observations that agents given i.c.v. in animals affect behavior, endocrine phenomena, and cerebral metabolism/blood flow parameters (Table II). Functional magnetic resonance mapping of neuroactive agents (e.g., the metabolically stable and potent NK1 receptor agonist GR-73632) introduced i.c.v. demonstrates rapid changes in the regional blood volumes of the amygdala, caudate putamen, and cortex (41). Thus, "pharmacological MRI" lends support to the paradigm that CSF-borne drugs exert effects on metabolism/blood flow in brain regions distant from the ventricles.

Many research models utilize the ventricular CSF as a "source" of the test agents that target particular brain loci. Consequently, to experimentally overcome the restrictive BCSFB and BBB, various water-soluble agents have been injected or infused i.c.v. to: 1) chemically ablate specific neuronal networks (42), 2) pharmacologically antagonize effects induced in certain brain regions by systemic drug administration (43), 3) minimize the development of distinct pathological processes, and 4) shed insight on mechanisms of drug action in the brain of various species. To provide perspective on this aspect of CNS pharmacology, a range of findings is presented in Table II regarding the use of CSF-delivered drugs to modify pain, seizures, amnesia, and Alzheimer's disease (AD)-like pathology (44–52). Exploiting the CSF for drug delivery to neuronal populations in animal models prompts consideration of the precise neuroanatomical relationships among the various CSF and brain regions.

DRUG TARGETS IN THE INTERIOR AND EXTERIOR OF THE BRAIN

CSF intimately contacts a number of regulatory or integrative centers in the walls of the ventricles and subarachnoid spaces. Drugs accessing the CSF can bind to receptors or modulate transporters near the internal and external surfaces of the brain. The periventricular and perimeningeal regions thus offer innumerable target sites for pharmacological exploitation. Table I summarizes salient features of

Table II. Alterations in Analgesic, Metabolic, and Pathophysiological Processes Following Intracerebroventricular Administration of Agents

CSF injectate	Species	Drug-induced actions on brain functions or disease states	Reference
2-Methyl-6-(phenylethynyl)pyridine (MPEP), 0.2 mg	Rat	Skin incision induced postoperative pain at 2 h was reduced by MPEP injected i.c.v. at the ED_{50}	(44)
Atosiban (oxytocin antagonist), [Cys(1)-D-Tyr(OEt)(2)-Thr(4)-Orn(8)]	Rat	Antinociception (evaluated by the trigemino-hypoglossal reflex) induced by oxytocin was abolished by the antagonist, atosiban	(45)
N3-Phenacyluridine, i.c.v., 0.5 μmol	Mouse	Antinociceptive effect of N3-Phenacyluridine (tail pinch analysis) was 1/50 that of morphine	(46)
Guanine-based purines injected at amounts of 300–640 nmol	Mouse	Seizures induced by the NMDA agonist and glutamate releaser, quinolinic acid, were partially abolished by GTP, GDP, and GMP	(47)
Fluoxetine injected bilaterally (i.c.v.), 0.015 mg	Rabbit	Theta oscillations in the hippocampus EEG were attenuated at least 50% by fluoxetine, a serotonin reuptake blocker	(48)
Spiroxatrine, a 5-HT1A antagonist, 0.005 mg	Rat	Retrograde amnesia, induced by the anesthetics midazolam and propofol, was completely antagonized by i.c.v. spiroxatrine	(49)
Humanin derivative, S14G-HN, i.c.v., 50 pmol	Mouse	Amnesia induced by β-amyloid25–35 was prevented by S14G-HN, as evaluated by the Y-maze test	(50)
Synthetic cannabinoid, WIN55, 212-2	Rat	Alzheimer's pathology (β-amyloid-induced microglial activation and cognitive impairment) was prevented by i.c.v. WIN55, 212-2	(51)
IgG1, IgG2a, and IgG2b isotypes of anti-Aβ	Mouse	β-Amyloid plaques in brain were reduced more effectively by y the IgG2 isotypes given i.c.v.	(52)

the various epithelia that interface the CSF with blood and brain. There are multiple downstream targets in CSF-bordering regions for agents that penetrate the CPs from blood or are otherwise introduced into the CSF system. The discussion below is arranged sequentially to correspond to the various proximal-to-distal sites along the ventriculo-cisternal–subarachnoid nexus.

Apical Membrane of Choroid Plexus

The choroidal apical membrane is on the central side of the BCSFB. It is therefore not usually accessible to water-soluble agents in plasma. Drugs that permeate the CP epithelium encounter a large array of receptors (e.g., for growth factors and neuropeptides) and transporters (e.g., for biogenic amines and organic acids) on the CSF-facing membrane. Modified transport activities of these apical membrane proteins can alter the CSF distribution of natural as well as xenobiotic compounds. The CSF level of amyloid precursor protein is altered by 5-HT_{2C} agonists such as dexnorfenfluramine (53). Stimulation of apical membrane 5-HT_{2C} receptors abounding in CP (54) might benefit AD by reducing beta amyloid (Aβ) production.

Another medical challenge, involving apical membrane transport phenomena, is to attain therapeutic concentrations of antibiotics in CSF. This is problematic because of the slow CSF-inward permeation of antibiotics across the barrier and the reverse, reabsorptive transport removing them from CSF (5). Agents are needed to specifically block the CP apical membrane reuptake of antibiotics from the ventricles. This would lead to greater bactericidal activity in CSF. On the other hand, it is desirable to *stimulate* reabsorptive transport by CP of certain molecules, e.g., Aβ, that when excessively present in CSF can harm the brain. Clearly, there are many opportunities for regulating CNS concentrations of drugs/metabolites, and CSF dynamics, by modifying transport processes at the CP apical membrane (55).

Ependyma

Ependymal cells are strategically located as "intermediary cells" between CSF and brain. A prominent characteristic of the ependyma lining the brain interior is the "leaky" gap junction. Gap junctions are permeable even to macromolecules. Test substances injected into the ventricular CSF distribute readily to the periventricular surfaces that are more permeable than the brain capillaries and choroidal epithelium. This renders the intracerebroventricular (i.c.v.) administration of agents a popular way to circumvent the BBB and BCSFB.

In addition to providing permeable "paracellular conduits" fostering molecular diffusion to and from brain, the ependymal cells have transporters that secrete and reabsorb substances from the ventricles. Ependymal cell receptor stimulation leads to a plethora of homeostatic activities. As an integral part of homeostatic responses, the ependyma help to mediate effects of growth factors (56), neuropeptides (57), proteins (58), cytokines (59), and hormones (60). These diverse modulatory actions benefit the neurons and glia. There is protection against focal cerebral ischemia, for example, when the IL-1 receptor antagonist is ependymally secreted into brain interstitial fluid and CSF (61). Ependymal cells also reflexly release peptides into the CSF. An example is the extrusion of acidic FGF into the lateral ventricles after feeding (62). The ependyma will likely prove useful as a drug target for controlling peptide uptake and release at the brain–CSF interface.

The ependymal wall and subependymal regions are also sites of malignant growth. These tumors require pharmacotherapy from the CSF side. Anticancer agents administered intraventricularly to circumvent the BCSFB are delivered by a single injection (63) or continuous perfusion with an Ommaya reservoir and catheter system (64). An important factor in regimens for introducing drugs into CSF is ventricular patency (65). Intraventricular methotrexate, for instance, can be fatal if CSF flow is severely obstructed (66). Perfusion chemotherapy involves infusion into a lateral ventricle and drainage from the temporal lobe or lumbar spine. This enables a high concentration of anticancer medication, say cytosine arabinoside, to be delivered briefly with minimal toxicity (64). Multiple Ommaya reservoir perfusions, dictated by recurrent tumors, have caused catheter infections and aseptic meningitis (67). Microbial and mechanical issues attending surgical invasion could be minimized by designing anticancer drugs for CP-mediated transport into CSF.

Subventricular Zone

With regard to stem cell therapy for neurodegeneration, the subventricular zone (SVZ) is of prime interest as a regulatory site. Lying just under the ependyma of the lateral ventricles, the extracellular matrix with its progenitor cells constitutes a "hotbed environment" for generating neurons. Precursor cells in the SVZ (germinal matrix) of fetal brain are converted to neurons for migration to distant sites of tissue construction. Certain stem cells in the *adult* SVZ are transformed as the result of mitogenic and neurotrophic modulation. This prompts searching for specific molecules, possibly conveyed by CSF, to promote neuron development in the disabled adult CNS (68). One neuropeptide showing promise in stimulating SVZ neurogenesis is pituitary adenylate cyclase activating peptide (PACAP). PACAP delivered i.c.v. promotes neural stem cell proliferation even in adult brain (69). Furthermore, secretin, a PACAP analog, is transported at the BCSFB by a saturable carrier (70). Enhanced transport of PACAP agonists by CP–CSF is therefore expected to augment neurogenesis in the SVZ.

Growth factors also regulate differentiation and growth of neurons. Basic FGF is one of several growth factors that regulates stem cell conversion in the SVZ. The CP abundantly supplies growth factors such as FGF2 to CSF and brain. In congenital hydrocephalus (71,72), when CSF flow is blocked and has altered composition there is a suppressed formation of neurons in the germinative zones (73). This indicates a relationship between CP–CSF growth factor availability and SVZ cell proliferation. Accordingly, CSF growth factor profiles favoring stem cell conversion to neurons should be identified. Such information would encourage pharmacological or transgenetic manipulation of CP–CSF growth factor titers in neurodegeneration to stimulate neuron production.

Circumventricular Organs

A dynamic exchange of solutes occurs between CSF and the circumventricular organs (CVOs). The CVO family of small neuroendocrine-type organs lines the walls of the third and fourth ventricles. CVOs receive fluid-borne signals such as thyroid hormones from both plasma and CSF (38). To integrate homeostatic activities for fluid and electrolyte balance, the CVOs also send neural signals to each other and the brain. CVOs include the pineal gland, subfornical organ (SFO), subcommissural organ (SCO), organum vasculosum of the laminar terminalis (OVLT), and the area postrema (AP). A distinguishing feature of each CVO is the permeable capillary bed at the core. Another characteristic is a peripheral ring of epithelial cells, the glia limitans, surrounding the neurons in all CVOs.

Epithelial cells encompassing each CVO are potential targets for CSF-borne drugs. Like the CP, the CVOs secrete products into CSF. The pineal gland and SCO, respectively, extrude melatonin and specific glycoproteins into ventricular CSF. Drug modulation of these secretions may be feasible. Decreased CSF melatonin levels are implicated in sleep dysfunction (74). Enhanced melatonin release into CSF might be accomplished by drug stimulation of pineal epithelial function. Another clinical problem involving CSF, i.e., congenital hydrocephalus, can result from disturbed secretion of SCO glycoproteins into the cerebral aqueduct (75,76). It is thus now evident that altered secretion of hormones and proteins into CSF creates behavioral and developmental problems. The pharmacological regulation of CSF glycoproteins may be the key to ameliorating some congenital disorders.

Another function subserved by the CVOs and CP tissues is drug metabolism. Hepatic-like activities of the epithelium comprising the ependymo–meningeal interfaces have been delineated by Ghersi-Egea *et al.* (2,77,78). The ability of the CSF transport interfaces to metabolize drugs and toxicants is an important "line of defense" for the brain. Critical "cleansing action" on the extracellular fluid results from the functionalization, conjugation, and extrusion phases of drug metabolism (79) in the CSF-bordering cells. Consequently, the level of oxidant compounds in CVOs and CPs is kept low to assure efficient homeostasis. When CVOs are inordinately stressed in aging and dementia, it may be helpful to boost the capacity of the CVO epithelia to detoxify harmful molecules.

Caudate–Putamen

Substances in the lateral ventricles readily diffuse across the permeable ependyma into the surrounding caudate–putamen regions. Tissue concentration profile data are used to quantify the rate and extent of solute penetration into periventricular tissue (80). ^{35}S-labeled phosphorothioate oligonucleotide (PS-ODN) intraparenchymal movement has been analyzed in Fischer 344 rat brain for up to 47 h (81). The calculated diffusion coefficients for PS-ODN decreased with time allowed for molecular spreading, suggesting that this nucleotide in transit was taken up by parenchymal cells or brain capillaries (81). Theoretically, pharmacological agents and micronutrients from CSF could diffuse a substantial distance within the caudate or hippocampus if not appreciably sequestered by brain cells or reabsorbed into blood. Low capillary permeability to interstitially migrating molecules, i.e., the chemotherapeutic cytosine arabinoside, minimizes molecular reabsorption into capillaries and thereby promotes intraparenchymal spreading of agents received from the CSF (82).

Overall then, many substances distribute throughout the brain by a combination of convection and diffusion to plasma membrane receptors. To a variable extent certain drugs are removed from the interstitial space by neurons, glia, or endothelial cells. However, this parenchymal cell uptake may also lead to a pharmacological action, e.g., steroid binding to nuclear receptors. Thus, in countless experiments the innumerable physiological responses to i.c.v. drug administration imply widespread permeation of test agents (Table II). The observations presented below exemplify how CSF-delivered drugs reach and affect neurons in the caudate nucleus and adjacent regions.

Time-course autoradiography tracks the CNS penetration routes taken by injected drugs. Remoxipride, an antipsychotic agent, is transported from the CP vascular bed to the CSF and thereafter to the interstitial fluid of the medial caudate nucleus, hippocampus, thalamus, and cerebellum. One hour after injection of ^{3}H-remoxipride, there is a gradient of radioactivity from the ventricles to deep regions of the cerebrum including the forebrain (83). This pattern of drug distribution from CP to CSF to brain is similar to that of vitamin C. Autoradiograms reveal that intravenous ^{14}C ascorbate rapidly penetrates the BCSFB (but not the BBB). Ascorbate then diffuses from the CSF (which acts as a source of substrate) to periventricular tissues and deeper regions (84,258). Both foreign and native compounds thus use the CP–CSF nexus to distribute into the caudate nucleus and putamen.

CSF perfusions and infusions are an effective way to evaluate test agent effects on caudate–putamen functions. Bilateral ventriculo-cisternal perfusion for several hours allows drug testing *vs.* vehicle control to be done concurrently on caudate regions in one hemisphere *vs.* the other. Bilateral perfusion in cats demonstrated histamine- and carbachol-induced increases in caudate blood flow that were blocked by cimetidine and atropine (85,86), respectively. In other ventriculo-cisternal perfusions testing for actions of alpha 2-receptor agonists in CSF, dexmedetomidine reduced cerebral blood flow in dogs by nearly 50% (87); the same experiments determined that ^{3}H-clonidine in CSF substantially penetrated the caudate nucleus. This convincingly reveals that CSF-distributed vasoactive agents induce substantial effects on blood flow to the caudate and proximate regions.

Long-term ventricular infusions permit other assessments of caudate functionality and responsiveness. Insight is needed on how the development of "preconditioning tolerance" attenuates infarction volume. Preconditioning with 20 min of focal ischemia significantly reduces major infarction to the caudate–putamen caused by 60 min of ischemia to the middle cerebral artery. Enhanced expression of bcl-2 in the caudate is implicated in this salutary effect (88). The hypothesis of bcl-2-induced neuroprotection was tested by infusing bcl-2 antisense oligodeoxynucleotides (ODNs) into a lateral ventricle for 3 days between the initial 20-min

preconditioning and the later 60-min major ischemic episode. Blocking of the tolerance by bcl-2 antisense ODN indicates a role for this antiapoptotic peptide in preconditioning (88). CSF delivery of ODNs are therefore experimentally useful for analyzing mechanisms of neuroprotection in the caudate–putamen.

Another potential CSF-oriented treatment of the caudate is for tumor suppression. The functional interactions between the caudate and adjacent CSF are significant in glioma spread and arrest with antitumor regimens. The therapeutic challenges encountered with human gliomas have prompted extensive modeling of experimentally produced gliomas in rodent caudate nuclei (89,90). Strategies involve both intracaudate (90) and i.c.v. presentation of agents. High-grade gliomas are difficult to treat, but radiosensitizing agents improve responses to irradiation (91–93). Rat glioma models show greater radiosensitization following exposure to acyclovir (92). An intraventricular route was used to infuse the radiosensitizer, 5-iodo-2-deoxyuridine (IUDR), to evaluate treatment of rat gliosarcoma (93). Tumor cells introduced into the caudate were more vulnerable to irradiation after exposure to IUDR by lateral ventricle infusion for 1 week (93). The combination of radiation and CSF-infused radiosensitizers requires investigation for tumor control in caudate and hippocampal regions.

Hippocampus

CSF pharmacokinetic principles that apply to the caudate also pertain to hippocampus. The CSF–hippocampus interface spans nearly the entire length of each lateral ventricle. Attention is being paid to hippocampal interactions with adjacent CSF during stroke and recovery (56,94–96). Hippocampus CA1 is especially vulnerable to transient forebrain ischemia (97). A host of growth factors and other neuroprotective agents given via CSF minimize untoward effects of stroke on rat hippocampus (68). Interleukin 3 (IL-3) infusion into the gerbil lateral ventricle for 1 week prevents delayed neuronal death in CA1 (98). This protection occurs through the enhanced postischemic expression of the IL-3 receptor alpha subunit in CA1. Other action mechanisms of CSF-delivered drugs have been analyzed in regard to hippocampal neuroprotection (99–102) at several phases of ischemia recovery (68).

Oxidative stress is a key factor in hippocampal degeneration. Chemical agents injected i.c.v. are useful "tools" to investigate stress responses to inflammation and neurotoxins. AF64A, a toxic analog of choline, interferes with choline transport thereby producing persistent hypofunction at cholinergic synapses. Injecting nanomolar AF64A into rat CSF heightens oxidative stress in the hippocampus and cortex (103). Light is shed on Alzheimer's disease and other cholinergic deficiencies when oxidative stress is studied coincidentally with acetylcholine disruption. Inflammation also generates oxidative stress. Fractalkine is a chemokine that controls inflammatory processes. Lipopolysaccharide (LPS) is a bacterial wall component experimentally used to activate the immune system and induce cytokine release. LPS injected i.c.v. induces inflammation and oxidative stress in rat hippocampus as reflected by increased 8-isoprostane levels (104). Administration of an antifractalkine antibody potentiates LPS effects, therefore implicating fractalkine as a neuroinflammation regulator. Such effects induced by i.c.v. injections reveal CSF utility in delivering agents to the hippocampus for the analysis of oxidative and inflammatory reactions.

Hypothalamus

Functionally and structurally, the CSF intimately connects with the hypothalamus. This compartmental proximity affords opportunities to modulate hypothalamic neuronal and epithelial systems with CSF-distributed drugs. Some peptides and hormones are circuitously transported from the blood to hypothalamus by way of the CP–CSF route (105). Prolactin is a prime example. It penetrates the blood–CSF interface (but not the BBB) by a CP carrier sensitive to plasma prolactin (106). Both the short and long forms of the prolactin receptor expressed in CP are implicated in saturable, receptor-mediated transport of prolactin from blood to CSF (107,108). Perturbed levels of plasma prolactin can injure multiple systems. Hypoprolactinemia, for example, harms immune functions, whereas hyperprolactinemia exacerbates systemic lupus erythematosus (109). It may be salient therefore to regulate prolactin transport at the CP. This would control prolactin entry into CSF and its delivery to "downstream" targets in hypothalamic nuclei. Altering a feedback loop that includes CP transport might regulate prolactin levels in CSF and plasma. Transport of other peptides should also be adjustable at the BCSFB to attain specific therapeutic goals (29).

Drugs and nutrients penetrate the hypothalamus via many distributional modes. Passive diffusion and bulk flow are common. There is also facilitated transport of proteins to the hypothalamus and nearby CVOs by tanycytes having heterogeneous functions (110). Tanycytes in the periventricular regions of the third ventricle have fibers that extend into the CSF. Accordingly, these neuroendocrine-like cells use their long fibrous extensions to transport peptides and drugs from the CSF to the hypothalamus. Thus, these tanycytic glia-like cells anatomically and functionally "bridge" the ventricular cavities with hypothalamic nuclei (111). Tanycytes express P-glycoprotein (P-gp) and the multidrug resistance protein (Mrp1). Both proteins actively clear various drugs and organic anions from CSF (5).

Altogether there are numerous transport mechanisms at the CSF–hypothalamus interface. Ependyma in certain regions of the third ventricle are linked by tight junctions. Here, endogenous solute fluxes into and out of the hypothalamus are regulated by active transporters. Consequently, the i.c.v. administration of peptides, antagonists, and drugs elicits many biochemical and functional responses in the arcuate, paraventricular, and supraoptic nuclei (112–121). Table III overviews endocrine investigations involving CSF drug delivery. Ventricularly injected agents induce rapid responses. Overall, there is substantial evidence that the hypothalamic nuclei are readily accessible to CSF-borne agents that modulate peptidergic systems. This suggests considerable potential in using CP–CSF drug delivery to modulate hypothalamic–hypophysial functions related to regulation of food intake, body weight, reproduction, sleep, and fluid balance.

Table III. Effects of CSF Injection or Infusion of Endocrine Substances on Hypothalamic–Pituitary Functions

CSF injectate	Species	Responses by cellular elements in the hypothalamus–pituitary axis	Reference
Neuropeptide Y; 10 mg for 30 min in lateral ventricle	Rat	Sevenfold increase in the number of CRH neurons in PVN with phosphorylated cAMP response element binding protein	(112)
Na-rich CSF infused into lateral ventricle for 14 days	Rat (Dahl)	Significant increase in ouabain-like compound in hypothalamus; Na-rich CSF increased blood pressure/heart rate dose-dependently	(113)
Endothelin (ET-1), i.c.v., 10 pmol in CSF	Rat	Paraventricular nucleus (PVN) function was required for ET-1 to exert pressor and AVP secretory effects	(114)
Leptin cDNA bolus injected into lateral ventricle	Rat	Ependyma-expressed leptin was secreted into CSF. Reduced food intake and body weight was due to stimulation of arcuate nucleus	(115)
Atrial natriuretic peptide; 1 mg for 30 min, i.c.v.	Rabbit	ANP significantly inhibited the EEG at 30 min in both the PVN and supraoptic nucleus (SON)	(116)
Neuropeptide Y; 12 mg/day for 4.5 days into third ventricle	Rat	NPY mRNA in arcuate nucleus was reduced by 50%, as a compensation to the induced overeating	(117)
Brain-derived neurotrophic factor; 5 μg	Rat	Arginine vasopressin mRNA signal was progressively decreased in parvocellular and magnocellular PVN regions of the hypothalamus	(118)
Genistein (phytoestrogen) infused into third ventricle	Sheep	Ovariectomized ewes displayed diminished GnRH stores in the median eminence and increased luteinizing hormone in the pituitary	(119)
Orexin A (1 nmol) infused i.c.v.	Rat	Orexin caused an increase in the wakefulness state, and a decrease in both the rapid and nonrapid eye movement sleep states	(120)
Bombesin (0.3 μg) infused in lateral ventricle	Rat	The anorexia induced by i.c.v. bombesin was completely prevented by a gastrin-releasing peptide receptor antagonist	(121)

Cerebellum and Brain Stem

At the brain's base, outlet channels or foramina connect the fourth ventricle CSF with the cisternal subarachnoid space. The cisterna magna serves as a convenient CSF sampling site. Cisternal CSF is a mix of fluid from the ventricles and basal subarachnoid space. Its composition represents the CSF system as a whole. Substantial pockets of CSF in various cisterns surrounding the cerebellum and hindbrain may act as drug "reservoirs." These large subarachnoid CSF pools should be factored into pharmacokinetic analyses. Drugs administered to the lateral ventricle reach the fourth ventricle and adjacent cisterna magna due to continuous CSF renewal and streaming down the neuraxis.

The posterior, ventral brain houses the fourth ventricle CP. Transport at this locus of the BCSFB affects metabolism of the cerebellum and the brain stem. Retinoic acid secreted by fourth ventricle CP, for example, regulates neurite outgrowth and cerebellar development (122). The fourth ventricle CP has specific temporal expression patterns for growth factor secretions that influence rhombencephalon development. Moreover, there are structural, functional, and pathological differences between the fourth and lateral ventricular plexuses (123–125). Regional variation holds promise for directed interventional strategies. Pharmacological and immunological analyses will likely identify regional CP differences to pinpoint sites of drug action. Specific manipulation of fourth ventricle tissue may minimize drug effects on the "upstream" lateral–third plexuses and nearby periventricular areas. CSF solute distribution originating in the fourth ventricle would convect agents to targets mainly (and in some cases, preferentially) at the exterior brain surface and meninges.

Arachnoid Membrane

CSF in the subarachnoid space is "sandwiched" between two thin membrane coverings: the arachnoid membrane apposed to the dura mater above and the pia–glia hugging the cortex below. Just as ventricular CSF furnishes beneficial materials to the brain interior, and collects harmful substances, so also the subarachnoid CSF acts as a solute "source" and "repository" for the CNS exterior. Figure 5 portrays anatomical relationships between the subarachnoid CSF and adjacent tissue compartments. The diverse and complex dynamics of brain–CSF molecular exchange has both pharmacological and toxicological significance.

At circumscribed locations in the arachnoid membrane, particularly close to the superior sagittal sinus, there are arachnoid villi "outpouchings" into the venous sinus. These valve-like structures in the CSF–venous blood interface allow egress of excess fluid and unneeded proteins, organic ions, and drug metabolites. With aging complications (126) and pathologic sequelae of hemorrhages in the CSF spaces (127,128), the resultant arachnoidal fibrosis reduces bulk-flow clearance of CSF from the CNS. Compromised CSF outflow creates physical (pressure) and biochemical (toxicity) problems for neurons. New agents are therefore needed to counter fibrosis effects on arachnoid drainage sites (129).

The arachnoid membrane is not a significant site for CSF formation. It does, however, manufacture and secrete proteins, cytokines, and prostaglandins similar to those in CP and ependyma (130). Cystatin C, a cysteine protease inhibitor, is expressed in arachnoidal and choroidal cells in humans (131). Cystatin C protects against cerebral ischemia (132). Somatostatin receptors 1 and 2 also localize to human CP and arachnoid (133). Their presence at these sites provides rationale for controlling fluid formation and reabsorption with somatostatin analogs that reduce CSF pressure in pseudotumor cerebri. Cardiotrophin-1 (CT-1) expression in the leptomeninges and CP is one of the few cytokines manufactured at the BCSFB (134). One postulate is that the CT-1-like protein prevalent in human CP and CSF diffuses to periventricular targets (134). Growth factors synthesized by the arachnoid are comparable to CP. Overall, the vast surface area of the arachnoid and underlying CSF is an underappreciated transport interface for regulating CNS drug distribution.

In the context of sleep regulation (135), prostaglandin D synthase (PGDS; or beta trace protein) has been extensively analyzed for its predominant expression in the arachnoid and CP (136,137). PGDS is undetectable in cerebral tissue but concentrated in meningeal-type membranes. Arachnoidal PGDS produces PGD2, which is somnogenic in mammals (135). PGD2 is secreted into CSF and carried by bulk flow to receptors in the ventrolateral preoptic area, a putative sleep center (138). The somnogenic effect of PGD2 is mediated by adenosine receptors. Tetravalent selenium inhibits PGDS and prevents sleep. Findings about meningeal PGDS/PGD2 and somnogenesis predict favorable pharmacological prospects for manipulating the arachnoid–CSF–brain nexus to regulate sleep.

Other therapeutic targets in leptomeninges are the multiple tumors that invade the arachnoid membrane. Leptomeningeal metastases are treated by intraventricular or intrathecal delivery of chemotherapeutic agents (67,139). Intrathecal methotrexate is widely used but has complications (139). By combining intrathecal and systemic methotrexate, along with other antitumor agents in alternate administration cycles, successful outcomes were obtained in patients with Burkitt lymphomas (140). The time to tumor progression is short. Other therapeutic modalities to treat CNS lymphomas are therefore indicated, including leptomeningeal targeting with antibodies. Rituximab is an anti-CD20 antibody that binds to cell surface molecules in B-cell neoplasms in lymphomas. There is no delayed toxicity in monkey recipients of intrathecal rituximab (141). Treatment of a refractory CNS lymphoma with rituximab, intravenously and intraventricularly, totally cleared tumor cells in CSF (142). CSF rituximab pharmacokinetics, however, needs further assessment.

It is worthwhile to evaluate specific CSF administration routes for rituximab and other antitumor agents. Is intrathecal drug delivery to leptomeningeal tumors, for example, as efficacious as intraventricular administration (143)? Alternatively, is there less toxicity after drug presentation to the "downstream" subarachnoid space because healthy "upstream" periventricular tissues are less exposed? In any case, CSF dynamics should be analyzed before intrathecal chemotherapy. Blocked CSF flow at a ventricular outlet, spinal subarachnoid compartment, or cortical convexity can be relieved by radiotherapy. Flow restoration prevents toxicity and tumor inaccessibility (144). Meningeal cancer therapy remains a challenge due to the dual impact of CSF bulk flow on tumor spread and treatment.

Pia Mater and Glia Limitans

The pia–glial boundary separating subarachnoid CSF from cortex exquisitely controls the disposition of molecules, microbes, and cells. Anatomically, the pia mater and subjacent glia limitans at the brain exterior are analogous to the ependyma and adjoining subependymal astrocytes in the CNS interior. Pia and glia perform secretory, reabsorptive, and metabolic functions that stabilize the brain interstitial fluid composition. Gap junctions in the superficial pial lining allow paracellular diffusional exchange of solutes between subarachnoid CSF and the underlying subpial space. However, the deeper, multilayered glia limitans restricts diffusion, thereby hindering solute penetration to the subjacent cortex.

Before describing the pia mater and glia limitans individually, it is instructive to describe how the pia–glial complex regulates trafficking of various-sized particles. Overall, drug penetration from subarachnoid CSF to cortical tissue is the net outcome of transport activity and permeability barriers. Although they permit access of many CSF-borne nutrients and drugs to brain, the pia mater and cortical glia limitans also "filter" out the larger toxins (145), pathogens, and cells that leak from blood into the subarachnoid space. In this way, the pia-glial lining is a physiological and structural "buffering" interface that protects neurons by not allowing CSF perturbations to be fully conveyed to brain interstitium. Inflammatory reactions such as meningitis, as well as hemorrhaging insults from subarachnoid bleeds, are thus largely confined to the CSF compartment (146). Isolating CSF reactions from the brain is crucial because excessive leukocytes, red cells, and platelets in the intrathecal space release cytokines that, if fully transmitted, would injure neurons. The "leptomeningeal defense" by the pia mater and astrocytic glia limitans thwarts parenchymal penetration of CSF-invading cells from the immune and vascular systems. Such subarachnoid containment explains why an infection such as bacterial meningitis is effectively managed by CSF-delivered antibiotics.

For pharmacokinetic reasons, it is useful to analyze components of the pia-glial complex of cell layers. The great permeability of the topmost pia mater permits penetration of large water-soluble agents. Vasoactive drugs injected intrathecally diffuse from subarachnoid CSF to the subpial space and modulate the caliber of vessels coursing through the leptomeninges. Cranial "window" preparations reveal leptomeningeal hemodynamic effects (147) and physiologic processes in the underlying glia limitans and cortex (148). Insight on such mechanisms is indispensable for designing agents to prevent debilitating cortical infarcts from compromised leptomeningeal perfusion. Drugs injected i.c.v. also access the subpial space (40). This is significant because drugs transported across the CP into ventricular CSF can gain access to the interior of the downstream leptomeninges.

Beneath the pia mater and subpial space lies the glia limitans (GL). It consists of several layers of astrocytic

elements. GL is an integral part of the massive, innermost leptomeningeal network of astrocyte processes covering the hemispheres and cord. Sheets of GL do more, however, than just physically separate pial tissue from brain (149). The GL has a critical role in fetal development to limit neurite extensions on the brain surface (150). In the adult CNS, the gap junctions between apposed astrocytes in the GL regulate ion and water fluxes over wide syncitia (151). Water channels such as aquaporin 4 are constitutively expressed near GL gap junctions (152). Orderly trafficking of molecules through astrocytic channels provides opportunities for pharmacological manipulation. Because aquaporin 4 and gap junctions facilitate water movement throughout brain, there is clinical interest to reduce cerebral edema (153) by downregulating aquaporin expression in the GL.

Plasticity of the GL has therapeutic implications. Expansion and contraction of astrocyte processes are normally part of neuroendocrine secretory phenomena in the hypothalamus (154). Astrocytes, including those in the GL, have supportive and protective roles in safeguarding neurons. To carry out these diverse functions, the GL must adapt to various states. It is desirable to seal membrane breaks when there is a breached GL barrier (150) leading to specific pathologies (155,156). With regard to strengthening the GL barrier, interleukin-1 and its receptor have been implicated in astrocyte reconstruction following traumatic injury (157). Conversely, in other instances it might be appropriate to temporarily increase GL permeability to enhance CSF drug uptake by brain parenchyma.

Moreover, pharmacological intervention to counter new GL formation may improve nerve fiber regeneration and migration at injury sites (158). Toward these ends two plasticity proteins for GL integrity have been identified: fukutin (159) and limitrin (160). Underexpressed fukutin or limitrin in the astrocyte feet disrupts the GL and attenuates its barrier properties (159,160). There is also toxicological interest in GL breakdown caused by intrauterine exposure to ethanol (161) or methylmercury (162). *In vitro* culture models simulate the interaction of GL astrocytes with other parenchymal elements (163). This allows assessment of factors that determine permeability. Consequently, it seems expedient to continue characterizing GL as a target for regulating molecular transfer across CSF–brain interfaces.

Subcortical Regions and Frontal Cortex

Subcortical and rostral regions of human brain are relatively far from the CP-ventricular system. This raises the issue of whether a CSF-transported drug can significantly reach distant parenchyma. Vitamin C is the prototype molecule actively transported into the ventricles and then widely distributed. The brain neither synthesizes vitamin C nor receives it across the BBB or arachnoid membrane (27). Neurons therefore depend upon a vitamin C supply mainly via the agency of the Na ascorbate cotransporter in CP epithelium (28). This cotransporter continually secretes ascorbate into CSF even in severe hypovitaminosis C. CP–CSF can thus constantly provide substantial amounts of vitamin C to neuronal networks.

Vitamin C delivery to brain via CSF occurs by diffusion and bulk flow. Solutes entrained in ventricular CSF contact a variety of "ports" along the ependymal wall. Here, drugs and nutrients enter the brain interior and then penetrate by diffusion (164). Substances (such as ascorbate) that diffuse from CSF to cerebral interstitial fluid more rapidly than they are subsequently taken up by more distal parenchyma and brain capillaries should thoroughly pervade the CNS. Bulk flow is another major factor in drug distribution throughout CNS compartments (165,166). Such volume transmission (39) occurs in extracellular compartments, especially around myelinated fiber tracts (167). This bulk flow extensively spreads drugs transported by CP, as well as lymphocytes that permeate the BCSFB (168).

Another "entry point" for CSF solutes is the gray matter on the exterior surfaces of the hemispheres, ventrally and dorsally. There, the more distal "recycling" of CSF into the Virchow–Robin spaces involves perivascular routes (35–37). Accordingly, a fraction of the CSF "recycles" by flowing from the subarachnoid space into the cerebral cortex alongside major vessels. Inward flow of CSF is promoted by arterial pulsations (35,36). Thereafter the "recycled" CSF is joined by interstitial fluid (formed at the BBB) before flowing outward along venous perivascular spaces back into CSF compartments (169).

Preferential flow routes for CSF also exist along white matter tracts deeper in the brain. These channels promote the low-resistance movement of fluid by bulk flow through brain intersticies (35,36,170). Bulk flow channels can be expanded by injecting fluid into brain parenchyma, thereby extending drug target range (171). Generally, the flow through extracellular channels boosts CSF circulation as well as solute distribution to subcortical and frontal regions (169). Still, there is a need for pharmacokinetic analyses to address how various types of drugs penetrate from the CSF into forebrain regions distant from potential entry sites at the blood–CSF interfacing regions.

Interregional Targeting by Transduction

Drug effects are related to factors other than distribution. A drug-induced action on neural transmission, for example, extends the sphere of an agent's influence on central functions. In other words, a CSF-borne ligand can affect outlying neurons relatively quickly without being convected over a long distance in the parenchyma. A solute's initial access to a CSF-bordering region (i.e., a CVO, hypothalamus, caudate–putamen, or hippocampus) demonstrates this point. Take the case of a CSF-carried peptide that binds to neuronal membranes in the subfornical organ (anterior wall of third ventricle) or is transported by tanycytes to specific hypothalamic nuclei (172). Such peptidergic stimulation of hypothalamic neurons generates electrical impulses that modulate fluid homeostasis through multiple neural projections to endocrine, autonomic, and behavioral areas (38). In another scenario, an agent in CSF readily gains access to neurons in hippocampal CA fields. As the result of drug action, impulses are generated by hippocampal neurons and propagated to thalamic nuclei to modulate a certain function. In both illustrations of putative pharmacological effects on neuronal systems distant from the CP–CSF, the agent supplied via CSF did not need to be transported to the affected locus. For some pharmacotherapeutic strategies, this

potential biochemical-to-neurophysiological "transduction" factor deserves consideration along with information about drug distribution to "distant" brain regions.

INTEGRATION OF DRUG-DELIVERY PATHWAYS IN THE CNS

A therapeutic drug level in a particular CNS area depends on transport and permeability factors operating simultaneously at the blood–CSF, blood–brain, brain–CSF, and CSF–venous interfaces. This review focuses on *brain-inward* solute transport across CSF-bordering regions. These mesenchymal support structures include CP, ependyma, pia–glia, and arachnoid membrane. Emphasis is placed here on the BCSFB because, along with the BBB endothelium, the choroidal epithelium seems highly exploitable for delivering blood-borne agents to the brain.

Blood–CSF Barrier *vs.* Blood–Brain Barrier

Choroidal drug delivery is especially relevant for targets near the main CSF circulation. These include the dozen or more regions, circumventricular and leptomeningeal, that were highlighted earlier. In clinical settings requiring sustained therapeutic concentration over larger areas of the brain, a worthwhile goal is to expedite drug delivery concurrently across the CP and BBB. The antiepileptic lamotrigine (173) and the neuroendocrine regulator leptin (105), respectively, represent water-soluble drugs and hormones that permeate both the blood–CSF and blood–brain interfaces. The strategy of "dual barrier" penetration would maintain brain drug concentration by minimizing CSF "sink action" on a water-soluble agent in interstitial fluid. Another way of decreasing CSF "sink action" is to inhibit CSF formation with an agent such as acetazolamide (23). Consequently, a drug slowly transported across cerebral capillaries into the interstitium is less likely swept away by attenuated CSF flow. With transiently reduced CSF turnover, therapeutic concentrations of hydrophilic drugs in brain might be effectively maintained. New paradigms can more closely examine the interaction between CSF dynamics and CNS drug distribution.

Choroid Plexus *vs.* Arachnoid Membrane

There are regional transport distinctions associated with the BCSFB. It is important to evaluate the properties of the CP and arachnoid membrane, respectively. Both interfaces engage in secretion and reabsorption, but with differing spectra of functional activities. Compared with the physiological appreciation of the CP (mainly fluid formation), there is a paucity of functional insight on the arachnoid membrane (largely fluid reabsorption). Yet it is known that the arachnoidal epithelial cells secrete a profile of proteins (i.e., growth factors, peptides, and enzymes) that matches the CP. This "common denominator" of protein production by the two epithelia affords the possibility for therapeutically achieving additive effects on brain cell targets. If the choroidal and arachnoidal secretions of a particular growth factor were pharmacologically augmented, e.g., following stroke or trauma (96,97,174,175), then even deeply located neurons might be repaired by neurotrophic protein conveyed dually by the ventricular and subarachnoid CSF supply routes (Fig. 1). Additional insight is needed concerning the role of the arachnoid membrane in CSF–brain pharmacokinetics. Molecular analyses of arachnoid *vs.* CP will reveal unique as well as comparable expressions of ligands and receptors. Such knowledge should spawn advances on receptor specificities in the various mesenchymal support structures at which to direct novel agents.

INNOVATIVE PHARMACO-MEDICAL APPLICATIONS TO THE CSF SYSTEM

Adjustments in CSF neurochemistry or drug content are central to remediating CNS disorders. Composition can be altered by modifying CP function or introducing therapeutic materials into specific regions of CSF. Utilization of animal CSF models has opened several avenues for new remedies. Translational pharmacology seeks to extend promising basic research to humans by achieving sustained resolution with minimal side effects and toxicity. CSF immunopharmacology, transplantations, gene therapy, phage display in CP, and CSF purification are novel dimensions with considerable potential for ameliorating brain diseases.

Bone Marrow Cell Infusions

CSF is replete with neurotrophic substances. A key question is whether supplemental trophic elements in CSF can alleviate neural damage. Bone marrow stromal cells (BMSCs) infused into fourth ventricle CSF are convected down the spinal cord. Following trauma induced by calibrated weight drop to the rat cord, infused autologous BMSCs attach to the spinal surface and invade the lesion site. BMSC infusion minimizes CSF cavitation and BBB rupture, thereby leading to improved behavior (176). Autologous transplantation to the CSF-cord axis would minimize immunostress in patients.

A significant challenge is to retain the CSF-infused BMSCs that disappear from original attachment sites in the cord after 3 weeks. Infused BMSCs probably secrete trophic factors into CSF at the injury locus, presumably accelerating repair (176). Moreover, patients with amyotrophic lateral sclerosis (ALS) undergoing spinal cord infusions with their own mesenchmyal stem cells (dissolved in autologous CSF) do not display altered cord volume or untoward cell proliferation rates (177). Such marrow treatments bypass the BBB via the CSF–cord routes. This promising infusion methodology may help to stabilize ALS and other spinal cord disorders.

Choroid Plexus Transplantation in Humans: Is It Feasible?

Epithelial cells have a proclivity for secreting growth factors. Omental tissue, transpositioned as an elongated pedicle from the abdomen to cerebral cortex, improves AD patient functions (178,179). Synthesis of BDNF, NT3/4, and NT5 by "transplanted" omentum (180) points to a choroid- or arachnoid-like effect in providing neurotrophic factors to a disabled brain. Transplanted CP engineered to abundantly

produce growth factors (32,68,96) could boost neural repair capacity. Cultured CP from infant mice is more potent than astrocytes in promoting neurite outgrowth from dorsal root ganglia *in vitro* (181). Moreover, grafting adult CP epithelium onto rat spinal cord facilitates regenerating axon growth in dorsal funiculus (182). Choroidal and other epithelial transplants are neurotrophic.

Encapsulation studies are also promising. Adult rat CP enclosed within alginate microcapsules and implanted in adult Wistar rat brains significantly reduces striatal infarct volume after middle cerebral artery occlusion (183). Encapsulated pig CP conferred neuroprotection in a rat stroke model, an effect likely due to choroidal secretions of GDNF, BDNF, and NGF (184). The ability of encapsulated CP to supply neurotrophic factors portends favorably for therapeutic transplants in neurodegenerative diseases (185). CP encapsulation in the ventricles needs refinement to avoid interfering with CSF dynamics.

Gene Transfer with Intraventricular and Intrathecal Vectors

Transgene material can be delivered via vectors to the CNS via three routes: the blood, CSF, or parenchyma. Vector administration to the ventricles or subarachnoid space is advantageous because CSF circulation promotes extensive distribution (186), thereby circumventing distribution barriers to agents in solid tissue. Commonly used CSF vectors are the adeno-, retro-, and lentiviruses. CP and ependyma display transgene expression (61) when the vector is injected into either brain parenchyma (187) or CSF. Systemically administered liposome "vectors" convey plasmid DNA to CP epithelium as well as cerebral microvessels (188). Mesenchymal support structures such as the arachnoid membrane (Table I) are therefore easily reached targets for vectors and DNA complexes presented by various modes and routes. Viral and liposome vectors also avoid compromised CSF flow caused by clustered vector-producer cells that proliferate inside the CNS (189).

Transduction manipulations differentially affect CNS regions. Expression depends on vector distribution and characteristics (190). In some experiments the CP epithelium is apparently the sole CNS structure expressing the administered transgene. Restricted cell type specificity for CP is uniquely exhibited by baculoviruses in gene transfer analyses (191). Other vectors bestow a greater range of CNS effects. With regard to a mutation affecting both CP and brain, e.g., the lysosomal storage disorder mucopolysaccharidosis I, the improved neuronal function following α-L-iduronidase transduction (192) is likely due to restored enzymatic viability of CP as well as brain parenchyma. Altogether, the findings indicate feasible strategies for directing vectors to the BCSFB.

Certain features of CP deserve attention in the context of CNS gene therapy and global distribution of protein products. Postnatal CP epithelial cells divide very slowly, making them suitable targets in adults for vectors that transduce well in nondividing or quiescent cells. The mitotic state of target cells, however, does not always predict response to transduction treatments (193). Lentiviral vectors appear promising for long-term expression in CP (194). Additionally, HIV-1-based lentiviral vectors can deliver genes encoding proteins fused to VP22, a tegument protein that mediates intercellular transport of proteins. This has implications for CP paracrine- or CSF-endocrine-like distributional mechanisms (194). Because the CP–CSF spreads secreted proteins widely, it is instructive to evaluate its role in synthesizing and distributing proteins such as tripeptidyl peptidase I (TPP-I). Mutated TPP-I, a lysosomal protease, causes late-infantile neuronal ceroid lipofuscinosis (LINCL). After intrastriatal injection of the TPP-I transgene into mice, there is expression in CP and ependyma as well as striatum (187). TPP-I immunostaining exceeds that of β-galactosidase, the vector distribution marker. This reveals extensive transport and uptake of secreted TPP-I (187). Pervasive distribution of the TPP-I transgene product bodes well for LINCL gene therapy. The CP's ability to express TPP-I and other transgenes injected into CSF suggests this epithelium as a locus for noninvasive pharmacological targeting in gene therapy.

CSF-instilled vectors can permeate many regions. Reporter gene expressions confirm that viruses efficiently transmit exogenous DNA to CSF-bordering regions (Table I) as well as large vessel adventitia (195,196); the latter indicates feasible gene targeting to Virchow–Robin spaces. Ependymal expression of β-glucuronidase (deficient in lysosomal storage disease) occurs after intraventricular gene transfer via a viral vector (197). The recombinant β-glucuronidase is secreted into CSF and subependymal regions from which its spread is enhanced by systemic mannitol-induced hyperosmolality (197). This distributional effect is linked to augmented flow of fluid through interstitial channels. It results from the imposed osmolality gradient between plasma and brain. Clinical usage of short-term osmolal imbalances may prove useful in promoting CNS distribution of transgene products or drugs.

In a leptomeninges tumor model, the CSF-administered gene for *LacZ* in a viral vector is taken up and expressed by ependyma, subependymal cells, and medulloblastoma, but not normal brain (198). Cancer cells on the cortical convexity are readily accessible to transgenes given noninvasively by way of indwelling CSF reservoirs in patients. Intrathecal instillation of transgenes may counteract other leptomeningeal pathology, such as the arachnoidal fibrosis that attends subarachnoid hemorrhage and normal-pressure hydrocephalus.

Several brain disorders in animal models have been rectified by CSF gene transfer (Table IV). Arterial spasm resulting from subarachnoid hemorrhage is attenuated by transgene-produced hemeoxygenase-1 (199). Stem cell conversion to neurons, potentially applicable to neurodegenerative diseases, is fostered by overexpressed BDNF (200) and FGF2 (201) stemming from exogenous growth factor genes introduced into CSF. Deleterious effects of cortical and hippocampal ischemia are lessened by HGF gene supplementation (202–204). Hearing loss induced by kanamycin is avoided by overexpressing HGF in spiral ganglion cells (205) via a CSF-distributed viral vector containing *hgf*. Because CSF is contiguous with inner ear fluid, there is the potential for intraventricular transgenes to repair ear damage.

CSF routing is favorable for gene transfer by viral vectors because it is relatively noninvasive and distributes "transgene products" efficiently. Overcoming CNS immune responses to vectors, and their inactivation by CSF, should advance virus application to brain gene therapy. Bacteriophages loaded with "therapeutic DNA" may also be used to

Table IV. Use of CSF Vectors in Gene Therapy of the Brain

CSF route	Species	Viral vector	Transgene(s)	Therapeutic or experimental effect	Reference
i.c.v.	Rat (SHR)	Adenovirus	*LacZ* and IL10	Cortical ischemia enhances expression of ependymal β-Gal and CSF [IL-10]	(195)
i.c.v.	Mouse	Adenovirus	β-Glucuronidase	Hyperosmolality augments distribution of ependymal glucuronidase	(197)
i.t.	Rat	HVJ-E	HGF	HGF overexpression prevents brain edema and BBB leakage after ischemia	(203)
i.t.	Rat	HVJ-E	HGF	Enhanced expression of HGF in spiral ganglion cells prevents hearing loss	(205)
i.t.	Gerbil	HVJ liposome	HGF	Transfected HGF gene prevents delayed neuronal death in CA-1 after ischemia	(204)
i.t.	Rat	HVJ liposome	HGF or VEGF	Elevated growth factor levels increase CBF and neuroprotection postischemia	(202)
i.t.	Rat	Adenovirus	HO-1	Increased expression of HO-1 in basilar artery reduces vasospasm after SAH	(199)
i.t.	Rabbit	Adenovirus	*LacZ*	Cation complexes with vector enhance β-Gal activity in arterial adventitia	(196)
i.c.v.	Rat	Adenovirus	BDNF	Overexpressed BDNF recruits new neurons even at nonneurogenic sites	(200)
i.c.v.	Gerbil	Adenovirus	FGF2	Augmented FGF2 levels promote great proliferation of progenitor cells	(201)

Vectors were injected into lateral ventricle (i.c.v.) or subarachnoid (thecal) space (i.t.).
HVJ-E, hemagglutinating virus-envelope; β-Gal, β-galactosidase; HO-1, heme-oxygenase; SAH, subarachnoid hemorrhage; IL, interleukin; HGF, hepatocyte growth factor; BDNF, brain-derived neurotrophic factor; FGF2, basic fibroblast growth factor; BBB, blood–brain barrier.

transduce CP epithelium (206). FGF2 and epidermal growth factor (EGF) ligands genetically displayed on bacteriophage coats (206,207) could bind to their cognate receptors on the CP cell surface (208) and enhance the expression of choroidal transgenic proteins for CSF distribution.

Virus and Leukocyte Trafficking Across Choroid Plexus: Implications for Pharmacotherapy

Penetration of immune cells and viruses across CP markedly affects CSF immunology and neuropathology. Pathogens readily infect the epithelium of the blood–CSF interface (209–211). Early therapeutic intervention might reduce the secondary harm to neurons caused by a virally devastated, dysfunctioning CP. Movements of viruses and inflammatory cells across CP into the CSF–brain are modulated by macrophage activity in the choroidal interstitium (212). Feline deficiency virus stimulates macrophages in CP, causing a toxic inflammatory response that spills into CSF (213). A compromised CP that adversely affects brain, even independently of viral loads, deserves investigation for drug-mediated restoration.

Regulated leukocyte traffic across CP is critical to CSF homeostasis and adaptation to disease and trauma (168,214,215). Monocyte penetration into the CP–CSF system attracts interest for medicopathological reasons. Challenges to the immune system lead to CP upregulation of mRNA for monocyte chemoattractant protein-1 (216). Accelerated monocyte trafficking into the CP–CSF–brain system has been associated with dementia (217). Notwithstanding the specific medicinal indications to slow down pathological trafficking of monocytes through the blood–CSF interface, it may also be possible to exploit monocyte permeation into CSF. Monocytes accumulate agents such as stavudine and abacavir (218). In antiretroviral treatments, peripheral monocytes that are transported across CP thus "carry" these drugs from plasma to the CSF–brain nexus. Consequently, monocytic leukocytes can be viewed as potential drug-delivery vehicles (218).

Phage Display of Peptide Ligands that Interact with Choroid Plexus

Medicinal chemistry and rational drug design have been challenged to overcome biological barriers to protein and peptide delivery to the CNS. A promising alternative uses combinatorial strategy with phage display for generating new peptide sequences that cross the CP and cerebral capillaries to access target cells in the brain. This process, which we call "medicinal biology," uses a tissue-innate biology to identify novel potential peptide biotherapeutics or alternatively transform known biotherapeutic peptides (e.g., growth factors) into drugs that can access the CNS by CP delivery and translocation into CSF. Identifying peptides that target CP will allow their attachment to potential biotherapeutic growth factors, for example, to direct them to CSF and hence the CNS. One such application involves steering plasma-borne growth factors such as FGF2 and EGF, by way of facilitated peptidergic transport across CP, into the CSF and nearby periventricular zones. Here one goal might be to stimulate stem cell conversion to new neurons for replenishing lost cells.

Phage display powerfully identifies ligands and their receptors that have the potential to distinguish normal *vs.* diseased CP. Highly complex combinatorial libraries (peptide, antibody, or cDNA ligands) can be effectively displayed on the phage surface (219–221). The physical link between the displayed protein ligand and its encoded DNA allows

the characterization of selected clones by sequencing the encoded DNA. Phage display screening has delineated a wide variety of protein–protein binding pairs (222). Once identified, second-generation phage-display libraries can select for alterations in affinity, specificity, or other features that define the ligand–target protein–protein interaction.

Traditional phage display biopanning is most effective when screened against purified target molecules. However, intact and cultured CP analyses can be limited by low receptor and ligand concentrations, higher background, and inactivation of functional phage particles.

Accordingly, to differentiate all surface interactions from functional cell surface binding, new methods are available (206,223): the selection of nucleic acid amplified phage (SNAAP) and ligand identification via expression (LIVE). The ultrasensitive SNAAP and LIVE methods (224) allow recovery of ligands normally lost by traditional biopanning. They also exploit the rational biology of the cell surface ligand–receptor interaction: internalization, organelle storage, and intracellular trafficking. Utilization of combinatorial techniques such as phage display should also reveal novel peptides biomarking CP epithelial cells. This will likely facilitate biotherapeutic targeting of CSF to treat various neurodegenerative diseases.

Regulation of Proinflammatory Cytokine Signal Dispersion Systems

Parenchymal cells at the blood–CSF interfaces carry receptors that respond to vascular immune signals (225). Immune system-to-brain signaling occurs when peripheral immune molecules interact with constitutively expressed CP receptors (e.g., CD14 and TLR4), thereby initiating cytokine cascades and signal transmission into the CNS (226). The so-called innate immune response (IIR) by the brain to circulating proinflammatory molecules begins at several barrier interfaces, including the CP and arachnoid membrane (Fig. 6). Upon initiation, the IIR "inflammatory wave" spreads by CSF volume transmission into deeper brain. Pharmacologic control of IIR depends on knowledge of the early stimulation of choroidal and meningeal receptors by peripheral cytokines. Other potential regulatory sites exist along the propagation routes traversed by barrier-generated "immune signals" (Fig. 6).

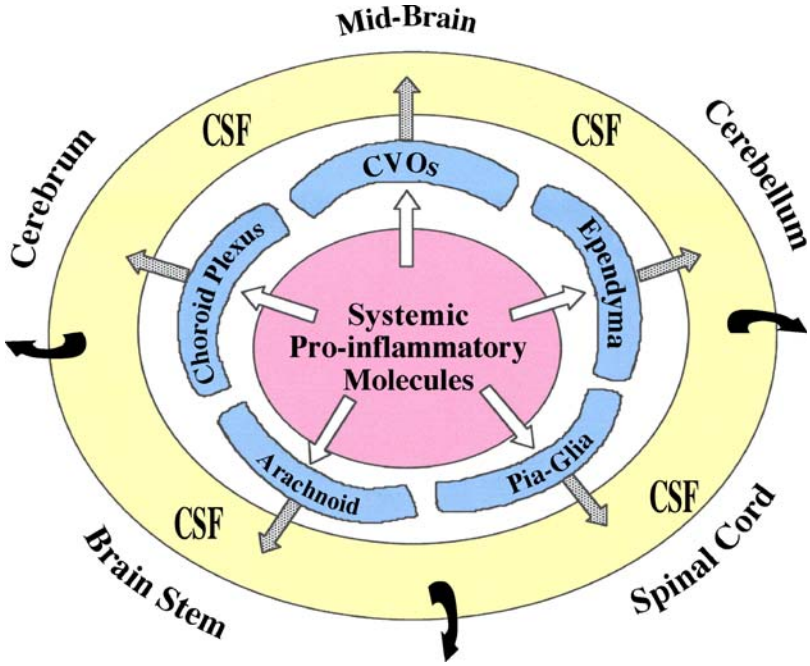

Fig. 6. Idealized schema for the reception and transduction of vascular immune signals for dispersal via CSF throughout the CNS: Cytokines and various pathogenic molecules in the blood diffuse through the CP capillaries and interstitium to activate receptors in the CP epithelial membrane (open arrows). This innate immune response to circulating proinflammatory molecules is initiated when peripheral immune molecules stimulate constituitive CD14 and TLR4 receptors in CP. Subsequently, molecules such as interleukin-1 are synthesized by CP and secreted into CSF (stippled arrows). The resultant 'inflammatory wave' of cytokines that is generated spreads by CSF volume transmission (bulk flow) into deeper parts of the brain (curved, filled arrows). The propagated cytokine signals may stimulate receptors in neurons, which can thereby effect adaptive responses to systemic pathogenic insults. Receptors in CSF-bordering regions [including CP, meningeal tissues, and circumventricular organs (CVOs)] are thus possible sites for regulating physiological cascades of cytokine fluxes into the CNS following infection and other peripheral inflammatory perturbations. Therefore the pharmacologic regulation of "inflammatory waves" to neuronal networks might be achieved by initially altering immune molecule interactions with cells at the blood–CSF interface.

The choroidomeningeal tissues and adjacent CSF act as a "functional bridge" to convert systemic biochemical information (associated with pathogenic insults) into central immune signals that stimulate downstream neuronal receptors. In this fashion, the CP–CSF system mediates the IIR that attempts to restore homeostasis perturbed by infection. Interleukin 1 (IL-1) has a major role. After injection of LPS to mimic bacterial invasion, there is upregulation of IL-1 mRNA in CP and a parallel increase in bioactive IL-1 concentration in CSF (227). This indicates that CP is activated by pathogenic molecules in blood, acts as a transducer to generate cytokine signals, and then transfers the latter to CSF.

A temporal analysis of c-*fos* expression is another manifestation of tissue response to stress, e.g., a pathogenic assault. Therefore it is instructive to analyze c-*fos* upregulation in CP in response to elevated peripheral cytokines. Following i.v. injection of the proinflammatory cytokine IL-1, there is c-*fos* mRNA labeling of CP and arachnoid by 30 min (228). Over the next 1–3 h, there is a second wave of activated c-*fos* that depends on molecules generated from the first-wave stimulation of CP by blood-borne IL-1. Transduced signals are propagated by diffusion and convection, eventually reaching CVOs, hypothalamus, and interior parenchyma (228). Excessive IIR responses can damage the brain. This raises the issue of designing agents to contain the IIR at its initiation sites in the BCSFB and BBB.

The IIR is driven through recognition systems such as CD14 and the toll receptors (TLR2 and TLR4) expressed in CP, arachnoid, and CVOs (229). Systemic injection of the bacterial endotoxin LPS releases proinflammatory cytokines from reactive macrophages and monocytes. LPS upregulates and binds to the CD14 and TLR4 receptors (230), thus starting cascades of immune signaling molecules in experimental animals. To date, most information about IIR has been deduced from descriptive expression patterns (spatial and temporal) in rodents injected with LPS. Kinetic analyses of tagged immune molecule trafficking from CP to CSF to brain, in animals challenged with pathogens, would elucidate adaptive mechanisms and their possible homeostatic significance for humans. Establishment of a major role for the CP–CSF in regulating central immune processes via IL-1, TLR, and CD14 receptors should prompt novel pharmacological interventions to better manage brain inflammations.

Countering CSF Neurotoxicity

CSF toxicity is a significant risk factor for brain malfunctions associated with central infections and degenerative diseases. CSF toxins disrupt basic neuronal functions. Immature neuronal networks are particularly susceptible to toxic molecules in CSF. Rat cortical neurons in culture exposed to HIV-infected CSF gradually lose their Ca-regulatory abilities (231). Cell death ensues. There is also dysfunction in cat neurons incubated with toxins generated by CP macrophages exposed to feline immunodeficiency virus (231). Developmental synaptic activity seems to render neurons vulnerable to CSF toxins stemming from CNS infections. This raises the question of neural stem cell sensitivity to CSF toxins produced in advanced aging and AD. Regimens promoting neural progenitor cell proliferation and differentiation will need to include measures for detoxifying the CSF of harmful metabolites arising from inflammatory reactions.

Gliotoxic factors are present in the CSF of multiple sclerosis (MS) patients (232). A 17-kDa glycoproteic factor in MS CSF kills cells of the BCSFB and BBB when it is injected into the rat CSF system. The demise of CP epithelium, ependyma, and arachnoid cells brought about by this toxic factor points to disrupted barrier systems as exacerbating the MS. Even 3 months after the injection of this human CSF toxin into the rat CSF, there was demyelination and TUNEL-positive dying oligodendrocytes (232). Extensive CSF biochemical profiles should be systematically acquired to identify specific toxic factors at various stages of degeneration in MS, AD, Parkinsonism, and Huntington's disease. Better management of CSF toxicity (by way of chelation, antibody neutralization, and perhaps dialysis) will likely lead to improvements in the cognition and affect of patients stricken with neurodegeneration.

CP–CSF ROLES IN TREATING NEUROLOGICAL DISORDERS

Conventional modes of drug therapy for neurological diseases have commonly used the BBB as the main site for delivering agents into the CNS. Although this strategy is certainly appropriate for many therapeutic situations, it is becoming increasingly evident that the CP–CSF interface may be specifically exploited for particular clinical needs. In other cases, it may be advantageous to identify agents that penetrate both the CP and the cerebral capillaries to achieve therapeutic concentrations of drugs that slowly penetrate both interfaces. To more fully realize multiple pharmacotherapeutic strategies, additional information needs to be compiled for drug actions and transport at the blood–CSF interface, and how these phenomena are altered by sundry neurological diseases.

AIDS and CSF HIV Phenomena

Productive infection of CP with HIV-1 occurs in humans (209). HIV-1 infects CP before it infiltrates the brain. Infection of the BCSFB may occur even before the onset of AIDS and immunosuppression. AIDS patients have a primary infection of HIV-1 in the monocytes and dendritic cells residing in CP. Findings from DNA analyses of HIV-1 strains in the human CP, spleen, and brain support the hypothesis that the blood–CSF interface is a port of entry for HIV-1 into the CNS (210). Furthermore, the investigation of an AIDS patient's HIV-1 gp160 genes in monocytes, CP, deep white matter, and lymph nodes provided sequence data to support the conclusion that HIV-1 permeates the CSF (which ultimately drains into lymph) (233). Experiments using radioactive virions demonstrated HIV-1 appearance in murine CSF and brain (234). This implies passage of HIV-1 across the CP as well as the BBB (234). Altogether, the pathological and molecular findings consistently point to CP involvement in the access of HIV-1 to brain. Accordingly, pharmacological strategies to thwart HIV-1 penetration

into the CNS must involve targeting of CP as well as the BBB.

Several studies have focused on the CP as a useful locus for delivering anti-AIDS agents to the CSF–brain nexus. Drugs have been targeted to HIV-1 loads in CP as well as brain. *In situ* perfusion experiments in guinea pigs reveal that various inhibitors of protease and reverse transcriptase (RT) differentially penetrate the CP, CSF, and brain compartments. The nucleoside RT inhibitor, 2′,3′-dideoxycytidine (ddC), penetrates CSF and brain slowly (235), because of its low lipophilicity and active reabsorption from CSF by organic anion transporters (OAT1 and OATP1). Another RT inhibitor, 2′,3′-dideoxyinosine (ddI), moves readily through the BBB and CP, the latter by an OATP2-like transporter that carries other nucleoside RT-inhibiting agents (236). Thus the RT inhibitors, ddC and ddI, have different characteristics in regard to their net transport from blood to CNS across the various barrier interfaces.

The nucleoside analog, lamivudine, is taken up by CP via a digoxin-sensitive transporter, but its permeation of CSF and brain is low (237). Zidovudine, another nucleoside analog, rapidly crosses the *in vitro* CP (to the CSF side) but is then actively taken back up by CP (238). Hydroxyurea is used in combination with nucleoside analogs. It is transported *in vivo* across both the BCSFB and BBB. However, hydroxyurea also is actively removed from brain by anion efflux systems that transport probenecid and digoxin (239). Collectively, these results emphasize the need for developing OAT protein inhibitors that will contribute to sustained therapeutic concentrations of anti-HIV agents in the CSF and brain.

The protease inhibitor, ritonavir, is actively accumulated by CP to an extent 25 times that of brain (240). The brain and CSF uptake of ritonavir is unaffected by OAT inhibitors, indicating that efflux mechanisms do not significantly affect CNS-wide distribution. The striking degree of uptake of ritonavir by CP points to carrier-mediated uptake. This is consistent with the ability of abacavir and nevirapine to competitively reduce ritonavir uptake and concentration at the blood–CSF interface. Overall, these pharmacokinetic analyses of anti-HIV agents point to pharmacological opportunities for targeting specifically located HIV burdens with drugs from various classes. Further delineation of distributional phenomena should enable regimens that more effectively prevent the emergence of intra-CNS reservoirs of HIV-resistant strains and cerebral impairment at late stages of infection (238).

Autoimmune Diseases and Infections

Inflammatory disorders in the brain are impacted by interactions of peripheral immune molecules and pathogens with CNS barrier systems. Consequently, there is both a choroid plexus component as well as a cerebral capillary arm that influence the course of central inflammatory responses. CNS inflammation can arise as the result of brain uptake of autoantibodies generated in the cervical lymph nodes (into which CSF drains) or as the result of attacks by viruses and bacteria. Low-level peripheral immune challenges, as for example with intravenous IL-1 or subseptic doses of lipopolysaccharide, induce tumor necrosis factor-α, IL-1, or c-*fos* in CP epithelium (227,228). Thus the parenchymal cells of the BCSFB are readily activated or primed to facilitate communication of peripheral immune signals to the CNS.

A recently proposed model emphasizes the blood–CSF interface as a major site for significant leukocyte movements (241). This allows extensive communication between the immune and central nervous systems. The CP epithelium normally allows lymphocytes and cytokine signals to be transferred bidirectionally between the periphery and CSF. Such leukcocyte trafficking and immune signaling are intensified in autoimmune diseases such as multiple sclerosis. In experimental allergic encephalitis (EAE), which has been used to model MS, there are marked changes in epithelial structure and adhesion molecule expression at the BCSFB (241). Immune surveillance of CSF and antigen-presenting activities by CP are also likely enhanced in response to CNS inflammation. The CSF thus serves as a dynamic medium for conveying immune signals and lymphocytes between blood and brain. CNS inflammation is regulated in part through adaptive changes in CP enzymes and adhesion molecules (241). Several molecules are implicated in the inflammatory responses at the BCSFB: matrix metalloproteinases (MMP), tissue inhibitors of MMP, cyclooxygenases (COX-1 and COX-2), and cellular adhesion molecules (ICAM-1 and VCAM-1). There are thus several protein targets in CP the pharmacological modulation of which may modify the course of brain inflammation. The delivery of anti-inflammatory cytokines to the CSF by nonreplicative viral vectors is a rising gene-therapy approach for MS (242).

Neurodegeneration and Stem Cells

Degenerative diseases, infections, ischemia, and trauma all take their toll on neuronal networks. Lost neurons need to be replaced through neuroregeneration involving progenitor or stem cells. Growth factors have a critical role in stem cell proliferation and differentiation within ventricular regions. A vital question is whether growth factor supplementation via CSF can expedite formation of new neurons in the SVZ and other neurogenic zones. An affirmative answer would suggest that neurodegenerative disorders could benefit from CSF growth factor therapy. Growth factors *in vitro* convert neural stem cells to clonal aggregates called neurospheres (243). Self-renewing, multipotential neurospheres exist in the walls of the lateral, third, and fourth ventricles, as well as in the cervical spinal cord around the CSF in the central canal (244). A worthwhile pharmacological goal is to regulate more efficient and sustained production of new neurons for the aged or injured CNS.

Epidermal growth factor (EGF) infused into the mouse lateral ventricle markedly expands the subependymal neural precursor cell populations (245). The exogenous EGF promotes proliferation and migration, causing nascent neurons and astrocytes to move away from the ventricular wall into brain parenchyma (245). FGF2 infused into the fourth ventricle of mice enhances the proliferation of neural progenitors around the fourth ventricle and spinal central canal. This proliferative effect is augmented when EGF is added to FGF2 infusion (244). However, the growth factor-induced proliferation in the fourth ventricle and cervical canal regions involves the formation of new glial cells only.

This is unlike the lateral ventricle subependyma where new neurons as well as glia are generated (245). Novel regimens of CSF growth factors may be feasible upon gaining additional insights on the neurogenic zones in the ventricular–central canal axis. This could stimulate new ideas on mechanisms for expanding the neuronal precursor populations close to CSF.

Human neural stem/progenitor cells (from a 9-week embryo) have been successfully transplanted, without immunosuppression, into the lateral ventricle of adult rats (246). The neural stem cell transplants remained viable for at least 4 weeks. Transplants in the form of neurospheres did not migrate well, because of the establishment of a surrounding glial barrier. Neural stem cells in suspension transplants, however, differentiated into neurons and migrated extensively (246). Another challenge in transplanting neural stem cells is to minimize their oncogenicity (247) while maximizing their ability to generate into new neurons with great migratory ability. There is a wide spectrum of neurogenic drugs that could be distributed by the CP–CSF to foster the development of fresh neurons. Neurotransmitters such as serotonin function as growth factors in regulating neurogenesis and neuronal survival (248). Agonists directed at serotonin receptors (5HT1C and 1A) show promise for acutely and chronically increasing the number of newly formed neurons in specific neurogenic zones (54).

Ischemia and Trauma

The CP is highly reactive to CNS damage incurred by stroke or physical harm (32). The blood–CSF interface promotes repair of itself and adjacent brain regions. Numerous growth factors synthesized and secreted in the CP–CSF system modulate reconstruction following ischemic damage to the plexus and periventricular zones (68). In addition, certain growth factors are transported from the plasma into the CNS (249). Intraventricular injections and infusions of growth factors and neurotrophic agents attenuate cell damage and death in hippocampus CA1 after experimental stroke induction (68). It thus seems worthwhile to consider therapeutic strategies to increase growth factor titers in patients with ischemia disorders. First, though, more information is needed to identify optimal combinations, concentrations, and timing of CSF growth factor boosting in animal models.

Although growth factors are nearly universal in their neuroprotectant or repairing potential, e.g., in regard to ischemia, trauma, and disease, there can be bothersome side effects from increased levels of certain factors. VEGF upregulation after cortical trauma in rats evidently contributes to brain edema (214). Moreover, excessive FGF2 in the ventricular system can be harmful to the ependyma and neurons in the subependymal zone (129). Clearly, further experimentation is indicated to ascertain growth factor regimens that avoid fluid imbalance problems and toxicity while maximizing optimal effects on neuronal networks near the CSF.

Hydrocephalus and Intracranial Pressure

CSF retention leads to ventriculomegaly and elevated intracranial pressure (ICP) in congenital hydrocephalus. Although acetazolamide is useful in acutely decreasing CSF formation and pressure, a new generation of agents is needed for sustained, long-term reductions in ICP. Neuropeptidergic agents may be useful for attaining better regulation of CSF dynamics (172). Receptors for arginine vasopressin (AVP) and atrial natriuretic peptide (ANP) in CP are targets for agents to reduce CSF formation (12,172). We have postulated that the NaK2Cl cotransporter at the CSF-facing membrane of CP is involved in peptidergic downregulation of CSF production (250). Thus the apical membrane of CP may be a regulatory site for feedback inhibition of CSF formation by neurotransmitters and neuropeptides when ICP rises in perinatal hydrocephalus.

Normal pressure hydrocephalus (NPH), or the more chronic type of hydrocephalus in the elderly, is often associated with compromised CSF reabsorption at venous drainage sites. Greater resistance to CSF outflow occurs when there is fibrosis in the arachnoid membrane, a condition brought on by excessive levels of FGF2 or TGFβ in the CSF (127–129). The development of antifibrotic agents (251) should be beneficial in managing CSF turnover problems caused by slower egress of CSF at the arachnoid–venous interfaces. An imbalance in growth factors such as FGF2 and TGFβ also alters fluid hydraulics at the BCSFB and in the brain interstices (252). More attention should be paid to the modulatory roles of FGF2 and TGFβ in brain fluid balance (253,254), and how this regulation is disturbed in NPH and age-related dementias.

Alzheimer's Disease

In advanced AD there are multiple functional failures in the CP–CSF nexus (32,255) as well as a breakdown of the damaged BBB. As a result, the profoundly altered composition of brain interstitial fluid compromises neurotransmission as well as neuronal metabolism. Harmful effects on the interstitium might be reduced by stabilizing the blood–CSF interface and the brain microvessels in early stages of AD (32). Stabilization would maintain CSF secretion as well as nutrient transport into the CNS. Exogenous growth factors and neuropeptides deserve more attention in the context of promoting brain fluid homeostasis (29). A central objective in AD pharmacotherapy is to enhance CSF turnover that is severely compromised (256). New agents are needed to augment CSF formation rate, thereby increasing CSF "sink action" on catabolites awaiting clearance from the interstitium. There is also a need for maintaining transporters such as LRP (257), in both the CP epithelium and cerebral capillary endothelium, to actively remove excess Aβ peptides from the CSF and brain interstitial fluid. AD dementia might very well be relieved by treatments that restore the deficient CSF dynamics and distorted neurochemistry in the elderly.

PERSPECTIVE AND PROSPECTIVE

The i.c.v. administration of bioactive agents has been the methodological hallmark of innumerable CNS pharmacological investigations. CSF-borne agents can penetrate rapidly (minutes to hours) and extensively into many CNS regions

(37,40,258). Consequently, multiple hundreds of drug actions in the brain have been elicited by experimentally injecting test agents (antagonists, peptides, growth factors, purines, ions, metals, enzymes, IgG isotypes, toxins, transgenes, etc.) into CSF to circumvent the BBB (examples are compiled in Tables II, III, and IV). It seems worthwhile to expand investigations for utilizing CSF as an access route to the brain and cord. Successful manipulation of the human BCSFB should provide more options for efficacious treatments of brain diseases.

Functionally, CSF has an "intimate relationship" with the brain, more so than blood. Pharmacological advantage should be taken of this situation. Effective pharmacotherapy is based upon sound appreciation of physiological principles. In this regard, evidence continues to mount that the CP–CSF system extensively mediates the transfer of hormonal signals, immune molecules, and trophic substances to the parenchyma of brain and cord. In yet other functions, all manner of unneeded catabolites, excessive ions, and potentially toxic peptides are actively reabsorbed by the CP to finely regulate the extracellular fluid composition. This myriad of transport activities supports the diverse functions carried out by neural parenchyma. Homeostatic disturbances in the diseased CSF and brain may be at least partially rectified by innovative pharmacological measures involving the CP and meninges.

Drug targets are embodied in the numerous transporters and synthesizing enzymes engaged in the vast trafficking of molecules by CP. These systems can be altered to therapeutic advantage for CSF–brain functions. Currently, there is an intense focus on the mechanisms of organic acid transport in the basolateral and apical membranes of CP (5,259). By manipulating these transport proteins, it should be possible to more effectively regulate the concentration of CSF opioid peptides (260) and other drugs (261,262).

CSF is accessible at a variety of choroidal and non-BBB extrachoroidal locations. Banks has posited the idea that CNS extracellular pathways (e.g., leaky vessels on the pial surface) are conduits for delivering some therapeutics (e.g., antibodies) to the CSF–brain nexus (263).

Certain epithelial transport proteins, receptors, and synthesized peptides not available for targeting at the BBB are extant at the blood–CSF interface. Moreover, unique characteristics of the tight junctions at these two main barriers (264) may provide opportunities for specific paracellular targeting. In some cases, the CSF may become the primary route/mode of therapy for certain brain disorders; in other situations, perhaps adjunctively with the BBB. The role of the CSF in CNS pharmacokinetics has recently been thoroughly addressed (265); and indeed, more comprehensive CSF sampling from patients will likely enable better diagnosis and management of disease.

A practical goal is to take advantage of the distinctive properties of both the BCSFB and the BBB. Given the extensive CSF involvement in disease progression, it is important to characterize the complex drug partitioning among the blood, CSF, and brain compartments. Due to the complexity of the CNS transport interfaces, conclusions drawn from CP–CSF pharmacology and toxicology data should advantageously include the combined use of *in vivo*, *in situ*, and *in vitro* models (1,6,266,267). On the pharmaceutical horizon are many encouraging prospects for regulating CSF drug delivery for the benefit of the brain.

ACKNOWLEDGMENTS

Concepts introduced or recapitulated in this article were stimulated by research funded by the Rhode Island Hospital (Lifespan), the Program in Neurosurgery at the Brown Medical School, the National Institutes of Health RO1 NS-27601 (CEJ), and BBSRC BB/C50466X (AB). We thank P. McMillan, A. Spangenberger, and V. Hovanesian for excellent electron microscopy and image analyses; and J. Johanson for graphical work. Our gratitude is also expressed to D. Smith for providing critique of the manuscript.

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