THE NEUROBIOLOGY OF GLIA IN THE CONTEXT OF WATER AND ION HOMEOSTASIS

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The brain is composed of two major cell types, neurons and glial cells. Though glial cells have been known for many years to be significantly more numerous than neurons in the CNS of vertebrates, current research has expanded our knowledge as to their important homeostatic and neuronal modulatory functions. Glial cells are divided into two major classes: microglia, which are phagocytes, and macroglia composed of oligodendrocytes, Schwann cells and astrocytes. Astrocytes are the most numerous glial cell type and account for one third of brain mass (Kandel, 1991). Astrocytes have irregularly shaped cell bodies and are characterized by an abundance of leaflet-like processes, which cover most synapses in the CNS. Some astrocytic processes form endfeet on the surface of the brain and spinal cord giving rise to a glial membrane (glia limitans), while other endfoot plaster along blood vessels forming a perivascular sheet (Fig. 1). This chapter will pertain mostly to astrocytes.

Astrocytes are involved in the maintenance of the blood–brain barrier (BBB), in the regulation of water and ion homeostasis and amino acid neurotransmitter metabolism, as well as energy and nutrient support of neurons. Maintenance of a stable internal osmotic environment is essential for normal cerebral activity. We will thus review current concepts with regard to the physiological properties of astrocytes and their control of water homeostasis.

SECTION I: NEUROTRANSMITTERS AND HORMONES

Glutamate uptake

L-Glutamate is the major excitatory amino acid in the brain. One of the major housekeeping tasks of astrocytes is to maintain a low concentration of extracellular glutamate (<1 μM). L-Glutamate uptake by astrocytes terminates its effects as a neurotransmitter, prevents extracellular L-glutamate levels from reaching excitotoxic levels, and acts as a metabolic substrate (Rothman and Olney, 1986; Choi, 1988; Sonnewald et al., 1997). A recent publication emphasized the importance of astrocytic coverage of excitatory synapses for glutamate clearance and thereby synaptic activity (Oliet et al., 2001).

High affinity glutamate transporters, GLT1 and GLAST1 subtypes, are enriched in astrocytic processes, and play the predominant role for glutamate clearance in the adult CNS (Rothstein et al., 1996; Rauen et al., 1998). Modulation of glutamate transporters may occur via slow a
regulatory mechanism or rapid regulation occurring within minutes (Davis et al., 1998; Duan et al., 1999; Munir et al., 2000; Poitry-Yamate et al., 2002). Glutamate transport is an energy demanding and electrogenic process by which one glutamate is cotransported with three Na\(^{+}\)/H\(^{+}\) (or two Na\(^{+}\)/H\(^{+}\) and one H\(^{+}\)/H\(^{+}\)) in exchange for one K\(^{+}\)/H\(^{+}\) and one OH\(^{-}\)/H\(^{+}\) (or one HCO\(_3^{-}\)/H\(^{+}\); Robinson and Dowd, 1997). The consequence of this stoichiometry is an increase in the Na\(^{+}\)/H\(^{+}\) concentration within the astrocytes, accompanied by an intracellular acidification and extracellular alkalization.

Within the astrocyte, glutamate is converted to glutamine through an ATP-requiring reaction catalyzed by the astrocyte-specific enzyme glutamine synthetase (Hallermayer et al., 1981; Fonnum, 1984; Loo et al., 1995). Glutamine is subsequently released to the extracellular space to fuel neurons and recycled into glutamate for glutamatergic neurotransmission.

**Glutamate signaling**

A variety of ionotropic glutamate (AMPA-kainate, glutamatergic N-methyl-D-aspartate [NMDA] and non-NMDA) receptors and metabotropic glutamate (mGlu) receptors have been characterized in glia in situ and in cultured astrocytes. The different cellular preparations differ with regard to expression of functional glutamate receptors, but it is clear that mGlu receptors (mGluR) mediate most biological effects (Sonnewald et al., 1997; Liao and Chen, 2003). Astrocytic mGluR are activated during neuronal activity (Poitry-Yamate et al., 2002) promoting the turnover of inositol triphosphate (IP\(_3\)), and triggering Ca\(^{2+}\) release from intracellular stores independently of extracellular Ca\(^{2+}\) (Cornell-Bell et al., 1990, 1992; Chiu and Kriegler, 1994; Steinhauser and Gallo, 1996). These events lead to Ca\(^{2+}\)-dependent glutamate release from astrocytes, which modulate synaptic activity. Thus, glutamate plays a central and bi-directional role in astrocyte–neuronal interactions (Haydon, 2001).

Cultured astrocytes express AMPA and kainate receptors (Gallo et al., 1989; Blankenfeld and Kettenmann, 1992) and, in response to ischemia in vivo and to anoxia in vitro, functional NMDA receptors (NR2B subunit) (Krebs et al., 2003). Further more, the colocalization in astrocytes of the NR2B subunits with NR1 subunits upregulates the expression of functional non-NMDA receptors (Krebs et al., 2003).

Recent studies have demonstrated that glutamate-mediated cytosolic Ca\(^{2+}\) oscillations regulate a pulsatile prostaglandin release from cultured astrocytes (Zonta et al., 2003b), providing a mechanism by which neuronal activity may influence vascular tone, the so-called functional hyperemia (Zonta et al., 2003a). In addition, glutamate induces the release from astrocytes of homocysteic acid, an endogenous agonist for NMDA receptors, through stimulation of both ionotropic (NMDA and non-NMDA) and mGluR (Benz et al., 2004). In this latter study, functional NMDA receptors on astrocytes are coupled to an intracellular Ca\(^{2+}\) increase via stimulation of the Na\(^{+}/\)Ca\(^{2+}\)-exchanger. Homocysteic acid release from astrocyte may thus contribute to the process of glia–neuron signaling.
Glutamate-induced cell swelling

Glutamate can induce astroglial cell swelling by interacting with mGluR. Relative changes in $[\text{Ca}^{2+}]_i$ can be detected simultaneously with changes in cell volume. Thus, $\text{Ca}^{2+}$ has been shown to be involved in glutamate-induced cell swelling (Koyoama et al., 1991; O’Connor and Kimelberg, 1993). Glial swelling induced by glutamate also results from the increase of intracellular osmotic activity due to uptake of $\text{Na}^+$ and glutamate (Schneider et al., 1992). The $\text{Na}^+$-$\text{K}^+$-$2\text{Cl}^-$ cotransporters, the $\text{Na}^+$-$\text{K}^+$-$\text{ATPase}$ pump, and the $\text{Na}^+$-dependent electrogenic glutamate carrier have also been implicated in this process.

Glial glutamate transporters, namely EAAT1 (GLAST) and EAAT2 (GLT-1) appear to be of primary importance in clearing the extrasynaptic glutamate content during glutamatergic activity (Danbolt, 1994; Schousboe, 2003). Neurons as well as the inotropic and mGlu analogs have been shown to induce the expression of EAAT1 and EAAT2 in astrocytes (Schousboe, 2003). The glial glutamate transporter GLT-1 appears to limit brain edema resulting from ischemia (Namura et al., 2002). The EAAT1 acts as a water channel and when expressed in Xenopus oocytes has a water permeability which is increased by up to 40% in the presence of glutamate (MacAulay et al., 2002). Thus EAAT1 may contribute to the regulation of water in the CNS. In fact, water loading-induced hypooosmotic stress in rats has been associated with an increased conversion in glial cells of glutamate to glutamine (Nagelhus et al., 1996).

Glutamate release mechanisms from astrocytes

Several astrocytic cytosolic glutamate release mechanisms have been identified. They include 1) the calcium-dependent release of cytosolic glutamate via exocytosis of...
Fig. 3. Ionic homeostasis in glia. Glial cells maintain the extracellular potassium ion (K⁺) concentration at levels which optimize neuronal function by actively taking up K⁺ from the extracellular space via activation of inwardly rectifying K⁺ channels, of K⁺Na⁺ pump catalyzed by Na⁺K⁺-ATPase, and of Na⁺K⁺Cl⁻ cotransporter. Excess intracellular K⁺ is shunted to the vascular system via outwardly rectifying K⁺ currents, Kir 4.1, Kv1.5, and rSloCa²⁺-activated K⁺ channel (RSloKCa), and swell-activated K⁺ currents during cellular RVD. Chloride ions (Cl⁻) are transported into the astrocyte by the Na⁺/K⁺/2Cl⁻ cotransporter and the Cl⁻/HCO₃⁻ anion exchanger. Several Cl⁻ channels have been described including inwardly and outwardly rectifying anion currents. The swell-activated Cl⁻ channels extrude Cl⁻ during the process of RVD. Sodium ions (Na⁺) channels in glia allow Na⁺ influx via the H⁺/Na⁺ antiport, and the Na⁺/K⁺/Cl⁻ cotransporter. Na⁺ extrusion occurs via the K⁺/Na⁺ pump and the 2Na⁺/Ca²⁺ exchanger. The efflux of HCO₃⁻ induces extracellular alkalinization and occurs via activation of the GABA A channels, the Na⁺/H⁺/HCO₃⁻ cotransporter, and the Cl⁻/HCO₃⁻ exchanger. Hydrogen ions (H⁺) are actively extruded across the cell membrane of astrocytes via the Na⁺/H⁺ antiport, the Cl⁻/HCO₃⁻ anion exchanger, and the Na⁺/HCO₃⁻ cotransporter. Astrocytes possess carbonic anhydrase, an isoenzyme that participates in water and electrolyte balance. The intracellular calcium ion (Ca²⁺) concentration increases following the release of Ca²⁺ from intracellular stores induced by ATP binding to the P2Y receptor, and following entry of Ca²⁺ through voltage-gated Ca²⁺ channels, and the 2Na⁺/Ca²⁺ exchanger.
glutamate containing vesicles (Bezzi et al., 2004; Kreft et al., 2004; Parpura et al., 2004); 2) the release of glutamate through sodium-dependent glutamate transporters operating in reverse mode (Rossi et al., 2000); 3) the release of glutamate via activation of VSOACs in the context of hypoosmolar stress and cellular volume regulatory response (Kimmelberg et al., 1990; Junankar and Kirk, 2000; Eggermont et al., 2001); 4) the release of glutamate via functional hemichannels (Ye et al., 2003; Parpura et al., 2004); and 5) the release of glutamate via purinergic receptors forming permeabilizing pores (Duan et al., 2003; Parpura et al., 2004).

A recent flurry of studies have analyzed the mechanisms of Ca\(^{2+}\)-dependent exocytotic release of glutamate. Glial Ca\(^{2+}\)-dependent glutamate release requires expression of several synaptic associated proteins, including synaptotagmin IV, a calcium binding membrane protein expressed in astrocytes. Vesicular glutamate transporters are critical for refilling of glutamate-containing vesicles in excitatory neurons, and may also play an equally important role in exocytotic glutamate release from astrocytes (Montana et al., 2004; Parpura et al., 2004). Interestingly, ATP released from astrocytes as a result of neuronal activity has been shown to, in return, modulate central synaptic transmission by suppressing glutamatergic synapses (Zhang et al., 2003), yet another example of the complexity of glia–neuron interregulation.

**Purinergic signaling**

Primary glial cells express several members of the P2Y purinergic receptor family. In situ, astrocytes express primarily P2Y2 and P2Y4 receptors (Simard et al., 2003). Purinergic P2Y receptors are expressed on astrocytes both in cell culture (Centeneri et al., 1997; Cotrina et al., 1998c; Fam et al., 2000) and in vivo (Franke et al., 2001; Zhu and Kimmelberg, 2001; Simard et al., 2003). Astrocytic Ca\(^{2+}\) waves in neonatal cortical astrocytes in culture are mediated by autocrine activation of purinergic receptors. In cultured rat astrocytes, ATP stimulates inositol phospholipid turnover, induces eicosanoid release and increases free [Ca\(^{2+}\)], (Brunner and Murphy, 1993). ATP triggers Ca\(^{2+}\) mobilization in primary cultures of rat neurohypophysial astrocytes due to PLC-mediated release from intracellular stores through activation of a pertussis-toxin-sensitive, G-protein-linked purinergic P2Y receptor (Troedec et al., 1999).

Although pharmacological studies from several groups suggested that long-distance Ca\(^{2+}\) waves are mediated by purinergic signaling (Cotrina et al., 1998c; Guthrie et al., 1999), it remained to be established how ATP was released and to which extent ATP release was regenerative. The fact that Cx-expression greatly enlarged Ca\(^{2+}\) waves led to the speculation that Cx hemichannels mediate ATP release. This suggestion was experimentally supported by the observation that several cell lines expressing Cx43, Cx32, or Cx26 released 5–20 fold more ATP than mock-transfected, Cx-deficient control cells (Cotrina et al., 1998b). In other words, Cx-expression enlarged Ca\(^{2+}\) waves by potentiating ATP release, rather than by increasing intercellular coupling. Gap junction channels, which are composed of two hemichannels from adjacent cells, have an inner pore diameter of 10–12 Å, and are freely permeable to large anions, including ADP and ATP. Open hemichannels may therefore direct the efflux of cytosolic ATP. Later studies documented that ATP release occurs concomitant with an increase in whole-cell current (Arcaino et al., 2002), supporting the concept of channel-mediated ATP release. Other lines of evidence have suggested, on the basis of cellular fractionation, that ATP is released by Ca\(^{2+}\)-dependent exocytosis of ATP-containing vesicles (Queiroz et al., 1999). Channel-mediated efflux versus vesicular release of ATP are not necessarily mutually exclusive mechanisms, and it is possible that they co-exist.

**HORMONAL REGULATION OF WATER AND IONIC HOMEOSTASIS IN ASTROCYTES**

Centrally released neuropeptides such as vasopressin (AVP), atriopeptin (ANP), angiotensinogen (AGT) and angiotensin (Ang) II appear to regulate fluid and ionic environment and cell volume in the CNS, possibly via intrinsic osmoregulation of glial cells.

**AVP**

The brain has a AVP-containing fiber system arising from AVP-containing neurons in the hypothalamus (Buijs, 1978; de Vries and Miller, 1998) and in vivo the extracellular AVP level may reach nanomolar levels (Robinson, 1983; Landgraf, 1992). V1 AVP receptors are distributed throughout the cerebral cortex (Chen et al., 1992; Brinton et al., 1994). Specifically, astrocytes express AVP receptors (Hosli et al., 1991) which appear to be of the V1b (Hertz et al., 2000) and the V1a subtypes (Katay et al., 1998).

AVP regulates water balance and stabilizes fluid osmolarity at the cellular level of the brain parenchyma (Fig. 4). AVP contributes to regulation of intracellular volume within the brain by regulation of water permeability in sub-pial astrocytes (Hertz et al., 2000). AVP also exerts effects on specialized astrocytes in the circumventricular organs and their adjacent glia limitans, and the neural pituitary, which regulate AVP release to the systemic circulation by altering the spatial relationship between neurons and their adjacent glial cells (Hertz et al., 2000). The effect of AVP on water permeability in astrocytes is assumed to be secondary to that of free cytosolic [Ca\(^{2+}\)] via the V1b receptor because the potency of AVP on water uptake is the same as that on [Ca\(^{2+}\)] (Safaraz and Fraser, 1999; Hertz et al., 2000). AVP treatment in brain astrocytes in culture appears to increase water space during RVD through a V1 receptor-mediated mechanism (Safaraz and Fraser, 1999). Latzkovits et al. (1993) have shown that AVP and ANP have opposing effects on glial cell volume. AVP increases while ANP decreases glial cell water content.

AVP causes a net increase in brain water content accompanied by a net electrolyte accumulation (DePasquale et al., 1989). AVP contributes to the control of [K\(^{-}\)] in brain by stimulation of K\(^{-}\) transfer from blood to brain, due to activation of an inwardly directed Na\(^{+}\)-K\(^{-}\), Cl\(^{-}\)transfer system.
cotransporter at the luminal membrane of capillary endothelial cells and opening of K⁺ channels at their abluminal membrane (Hertz et al., 2000). Local AVP release in brain tissue is increased by high K⁺ levels (Landgraf, 1992). AVP further increases the water content of astrocytes following K⁺-induced swelling by inducing the uptake of Na⁺, K⁺, and Cl⁻ together with some osmotically obliged water via the cotransporter and/or channel-mediated uptake of KCL (Hertz et al., 2000). AVP increases Cl⁻ uptake in type-1 astrocytes in culture in a dose-dependent manner, with a significant effect at concentrations greater than 10⁻⁹ M. This AVP-dependent increase is inhibited by both a V1A AVP receptor antagonist and bumetanide (inhibitor of Na⁺-K⁺-2Cl⁻ cotransporter), indicating that the peptide-mediated increase is V1A receptor mediated and involves the cotransporter (Katay et al., 1998). Thus, Cl⁻ homeostasis in the neuronal microenvironment may be regulated by the effect of AVP on astroglia (Katay et al., 1998). AVP and V1 receptors thus play a crucial role in the regulation of brain water and ion homeostasis though the effect of AVP on aquaporin-mediated water flux through astrocyte plasma membranes remains to be proven.

Fig. 4. The AVP–ANP system in glia. AVP has been demonstrated to induce water and cellular ionic homeostasis in astrocytes via the V1 receptor. AVP activates the Na⁺/K⁺/Cl⁻ cotransporter thus increasing water content in astrocytes by activating the uptake of Na⁺, K⁺, and Cl⁻. AVP has been shown to upregulate the expression of AQP4. ANP decreases Cl⁻ uptake in astrocytes.
(Holthoff and Witte, 2000; Niermann et al., 2001). Thus far, scientific evidence in favor of aquaporin modulation by AVP includes the demonstrations of 1) the facilitation by AVP and AVP receptor agonists of a radial water flux in the rat neocortex following neuronal activity (Niermann et al., 2001) and 2) the immunofluorescence of rat cortical slices treated with AVP revealing intense AQPF immunolabelling associated with the pial surface and blood vessels (Niermann et al., 2001).

**ANG**

C-type natriuretic peptide is a member of the ANP family. The actions of ANP are generally opposite to those of angiotensin (Ang) II. This peptide is considered to play an important role in the regulation of water and sodium in the body by its abilities of natriuresis, diuresis and vasorelaxation (Wilkins et al., 1997). ANP also inhibits drinking behavior. C-type natriuretic peptide is expressed in brain where it is thought to act as a local paracrine hormone (Barr et al., 1996; Fig. 4). In astrocytes cytosolic Ca^{2+} oscillations appear to control the release of ANP stored in a distinct population of vesicles suggesting a role in gliaendothelia signaling (Kreft et al., 2004). ANP modulates unidirectional transport of sodium from blood to brain via the cGMP–PKG pathway by inhibiting amloride-sensitive Na^{+}/H^{+} exchange (Ennis et al., 1996; Ibaragi et al., 1989). ANP administration exacerbates brain edema after the experimental cortical contusion of rats, possibly because of an increase in the BBB permeability via the cGMP pathway, whereas it reduces brain sodium levels (Fukui et al., 2003). In type-I astrocytes, it has been shown that ANP decreased Cl^{-} uptake in a dose-dependent manner, this effect being abolished by AVP (Katay et al., 1998).

**Renin–Ang system (RAS)**

The precursor decapeptide molecule AGT, a glycoprotein of molecular weight 55,000–58,000, is converted by renin into Ang I. Ang I is converted by Ang converting enzyme to Ang II which is known to regulate blood pressure and body fluid homeostasis through its action on the Ang 1 (At1)-receptor.

The brain contains an autonomous RAS (Alliot et al., 1999a; Davisson, 2003) of which astrocytes appear to be the primary source of AGT (Stornetta et al., 1988; Fig. 5). Large amounts of AGT mRNA are found in the forebrain and medulla of the rat, with astrocytes being the chief source (Stornetta et al., 1988). AGT mRNA is also expressed in human astrocytes (Ferrario et al., 1991). Transgenic mouse models containing human AGT genomic transgenes have been used to identify the localization of AGT to astrocytes in almost all brain areas (Yang et al., 1999). Where AGT is processed once it is synthesized and what other peptides are formed remain uncertain (Fitzsimon, 1998). TTF-1, a homeodomain-containing transcription factor, participates in the control of body fluid homeostasis by regulating AGT gene transcription in the rat subfornical organ (June Son et al., 2003). Glucocorticoid and mineralocorticoid treatment causes increased in AGT mRNA in presumed astrocytes in the anterior hypothalamic, POA, PNV, SON, suprachiasmatic nucleus, and periventricular nucleus, where as adrenalec tomy had the reverse effect (Ryan et al., 1997). However, glucocorticoids inhibit AGT secretion in rat C6 glioma cells (Sernia and Thomas, 1994). A novel transgenic rat model expressing ANG antisesse under the control of a glial-specific promoter exhibited reduced dipsogenesis to i.c.v. infusion of renin, blunted physiological responses to salt loading, increased sensitivity to the pressor effects of Ang II injected into the rostral ventrolateral medulla (Baltatu et al., 2001a), and heart rate variability as well as alterations in blood pressure (Baltatu et al., 2001b).

The presence of At1-receptors on perivascular astrocytes suggests that Ang may act on astrocytes in an autocrine or paracrine manner (Alliot et al., 1999a). Using in situ hybridization and reverse transcriptase–polymerase chain reaction, Chen et al. (2003), verified that Ang At1a and At1b receptors are expressed in mouse brain in areas involved in osmotic regulation and in the pituitary. Ang II receptors expressed on distinct populations of neonatal rat astrocytes may activate unique signaling pathways and produce different cellular responses. Three different populations have been described: astrocytes containing Ang II receptors of the At1 subtype coupled to PLC activation and prostacyclin release (in the medulla and cerebellum); astrocytes containing high affinity Ang II receptors which do not activate PLC or release prostacyclin but may be coupled to an additional signaling pathway (in cerebral cortex); and astrocytes which do not contain high affinity plasma membrane Ang II receptors and do not activate PLC or release prostacyclin (in thalamic/hypothalamic area; Tallant and Higson, 1999). Ang II has been shown to induce Ca^{2+} signaling coupled to the expression of the At1 receptor subtype in astrocyte cell cultures derived from the neonatal rat circumventricular organ (Gebke et al., 1998).

The presence of immunoreactivity to Ang I and II around most brain vessels is described in mice brain tissue. Ang are present at the plasma membrane of brain parenchymal cells, presumably in perivascular astrocytes which are also immunoreactive to At1-receptor antibodies. Ang I, and II have been localized at the surface of astroglial endfeet around blood vessels, in very close proximity to aminopeptidases A and N, which play an important role in the Ang metabolism (Alliot et al., 1999a,b). These aminopeptidases are expressed in the abluminal face of pericytes and periendothelial cells (Alliot et al., 1999b). Such findings suggest the presence of a distinct perivascular Ang system in the brain which may have a distinct role in cerebral blood flow regulation (Alliot et al., 1999a).

How Ang II is produced in the CNS and the precise mechanism of action of this complex RAS in the brain remains unresolved. Whether Ang peptides derived from brain act as neurotransmitters or exercise a paracrine role remains to be determined (Fitzsimon, 1998). Most experimental evidence supports the view that brain Ang II is important in the regulation of blood pressure, sodium homeostasis, and blood volume (Fitzsimon, 1998) by increasing sympathetic outflow,
AVP release, water intake, and salt appetite (Wright and Harding, 1992). Ang may cause thirst by inducing increases in the extracellular sodium concentration in the brain (Blair-West et al., 1994). Transgenic mice overexpressing both human renin and human Ang II in glia were moderately hypertensive and exhibited an increase in drinking volume and salt preference, suggesting that chronic overexpression of renin and Ang II locally in the brain can result in hypertension and alterations in body fluid homeostasis (Morimoto et al., 2003).

Fig. 5. The RAS in glia. Astrocytes are the primary source of ATG in the brain. TTF-1, a homeodomain-containing transcription factor, participates in the control of body fluid homeostasis by regulating AGT gene transcription. Ang I and II are detected in perivascular astrocytes which are also immunoreactive for the At-1 receptor, the binding site for Ang II. Ang II has been shown to induce Ca²⁺ signaling coupled to the expression of the At-1 receptor.
Glucocorticoids

Gial cells are the target for glucocorticoid control of gene transcription in the CNS (Vielkind et al., 1990). The distribution of type II glucocorticoid receptor (GR) immunoreactivity is widespread in gial cells throughout the brain (McGimsey et al., 1991; Ahima and Harlan, 1990; Cintra et al., 1994; Joels and de Kloet, 1994). GRs typically act by binding to specific DNA sequences, the glucocorticoid response element, to affect transcription (Martinez and Wahli, 1991). GRs can bind to components of the AP-1 complex, formed by homodimers or heterodimers of the protooncogene proteins c-fos and c-jun and thereby inhibit genes that are normally activated by the AP-1 transcription factor (Yang-Yen et al., 1990). Glucocorticoids potentiate astrocytic signaling via long-range Ca2+ waves and enhance intracellular Ca2+ mobilization and ATP release in rat astrocytic cultures via GR signaling (Simard et al., 1999).

Glucocorticoid treatment in rats reduced gial fibrillary acidic protein (GFAP) transcription and increased glutamine synthetase transcription in hippocampal tissues (Laping et al., 1994) and in gial cells (Juurlink et al., 1981; O’Banion et al., 1994). Astrocyte proliferation appears to be in part regulated by alterations in GR pathways (Crossin et al., 1997).

Steroid actions upon gial may affect neighboring neural function. Steroids such as dexamethasone, hydrocortisone and progesterone have been shown to inhibit C6 astroglial cell-induced microvesel morphogenesis (bovine retinal microvascular endothelial cell differentiation into capillary-like structures) via a receptor-mediated mechanism (Wolff et al., 1992). Glucocorticoids may also modulate the formation of intramembrane orthogonally packed aggregates of particles. Dexamethasone, a potent glucocorticoid, affects the differentiation of neonatal rat astrocyte membrane structure into two patterns depending on the rate of proliferation in cell culture. In confluent secondary cultures, dexamethasone exposure increases the density of orthogonally packed aggregates of intramembrane particles forming assemblies up to values present in gial limits in vivo. In rapidly proliferating astocytes, during the first week of secondary culture, most astocytes exposed to dexamethasone transiently failed to express assemblies, until the time they reached confluency (Landis et al., 1991).

Also, gial can metabolize steroid hormones and can manufacture both cholesterol and pregnenolone, the first steps in the biosynthesis of all steroids (Jordan, 1999).

SECTION II: CALCIUM SIGNALING

Ca2+ signaling in astocytes

Ca2+ signaling plays a central role in cell activation. Ca2+ is a second messenger responsible for the regulation of a wide range of cellular processes (Berridge, 1994). Astrocytic Ca2+ signaling is expressed as oscillations in cytosolic Ca2+ concentrations ([Ca2+]i) and as slowly propagating waves of [Ca2+]i increments (Smith, 1994). Astrocytes display regular oscillations when activated by stimuli including hormones.

In addition to Ca2+ oscillations, astrocytes also actively propagate Ca2+ waves. Mechanical stimulation of single astocytes resulted in expanding waves of [Ca2+]i increments, which engaged 20–100 neighboring astocytes in culture (Charles et al., 1991; Smith, 1994). Cornell-Bell and co-workers (1990) were the first to describe that astocytes generated propagating Ca2+ waves when exposed to glutamate. The discovery that astocytes possess a mechanism for long-distance communication had a profound impact on the field and questioned the view that astocytes are passive support cells. Later, studies showed that astrocytic Ca2+ waves evoked large spike-like increases in neuronal cytosolic Ca2+, suggesting that astrocytes may participate directly in neurotransmission (Nedergaard, 1994; Parpura et al., 1994). It was subsequently confirmed that astrocytic Ca2+ signaling modulates the strength of synaptic transmission: stimulation of astocytes led to a decrease in synaptic failure rate between pairs of synaptically coupled interneurons and CA1 pyramidal cells (Kang et al., 1998). Astrocytic Ca2+ waves modulate the firing frequency of both ganglion cells in dissected eye cup retinas and in hippocampal cultures (Newman and Zahs, 1998). Similarly, Schwann cells regulate neuromuscular transmission via a pathway that requires intracellular Ca2+ signaling (Robitaille, 1998). Together these observations identified a new signaling loop between neurons and astrocytes: astrocytes can modulate the Ca2+ level, and thereby the firing pattern, of neurons in their surroundings. In turn, neurons can trigger astrocytic Ca2+ signaling by releasing glutamate.

In rat astocytes, in vivo, initiation and propagation of Ca2+ waves involve a sequence of intra- and intercellular steps in which purinergic receptors, gap junctions Cx43, PLC, IP3, and internal Ca2+ stores, all play a role (Venance et al., 1997; Cotrina et al., 1998b; Guthrie et al., 1999; Arcuino et al., 2002). The cytoskeletal organization is also a prerequisite for interastrocytic Ca2+ signaling mediated by the release of ATP (Cotrina et al., 1998c). Calcium in of itself appears to have a role in triggering Ca2+ release from IP3 sensitive stores in astocytes during the generation of spontaneous [Ca2+]i oscillations (Parri and Crunelli, 2003).

Astocytes express multiple types of voltage-gated Ca2+ channels supporting the notion of complexity with regards to Ca2+ homeostasis in this cell type (Latour et al., 2003). The extracellular accumulation of K+ resulting from neuronal high-frequency stimulation induces Ca2+ influx into astocytes through voltage-gated Ca2+ channels and
Lowering extracellular Ca\(^{2+}\) triggers astrocytic Ca\(^{2+}\) signaling

Low [Ca\(^{2+}\)]\(_{o}\) induces [Ca\(^{2+}\)]\(_{i}\), release that include single cell Ca\(^{2+}\) oscillations and propagated intercellular Ca\(^{2+}\) waves (Zanotti and Charles, 1997). This response is abolished by thapsigargin (inhibitor of IP\(_3\)-sensitive Ca\(^{2+}\) stores) and by the phospholipase C antagonist U73122 and increased following application of ATP to glial cell cultures (Zanotti and Charles, 1997). A component of the astrocyte [Ca\(^{2+}\)]\(_{i}\) response also appears to be mediated by extracellular Ca\(^{2+}\) entry through voltage-gated Ca\(^{2+}\) channels (Barres et al., 1990; MacVicar et al., 1991). The Na\(^{+}/Ca\(^{2+}\) exchanger protein and mRNA have been detected in astrocytes (Goldman et al., 1994). An ATP-driven Ca\(^{2+}\) pump and the Na\(^{+}/Ca\(^{2+}\) exchanger mediate Ca\(^{2+}\) influx into the cell upon reduction of the Na\(^{+}\) electrochemical gradient. Thus, lowering [Na\(^{+}\)]\(_{o}\) has been shown to cause [Ca\(^{2+}\)]\(_{i}\), to rise (Goldman et al., 1994).

Astrocytes have been shown to release glutamate in response to lowered [Ca\(^{2+}\)]\(_{o}\) (Jeftinija et al., 1996). This effect could be mediated by an increase in cytosolic Ca\(^{2+}\) in accordance with the Ca\(^{2+}\)-dependent mechanism for glial release of glutamate that has been proposed by Parpura (Parpura et al., 1994; Zanotti and Charles, 1997). A decrease in [Ca\(^{2+}\)]\(_{o}\) has been observed in vivo under multiple pathological conditions including ischemia (Meyer, 1989; Silver and Erecińska, 1992; Kristian et al., 1996) and hypoglycemia (Kristian et al., 1996). Under these pathological conditions, [Ca\(^{2+}\)]\(_{o}\) has been shown to fall to 0.01-0.1 mM, a concentration range that consistently evokes glial signaling (Zanotti and Charles, 1997). There is also evidence that low [Ca\(^{2+}\)]\(_{o}\) plays a role in the initiation of seizures. Clinically, hypocalcaemia is associated with seizure activity. In an animal model, a decrease in [Ca\(^{2+}\)]\(_{o}\) has been observed at the onset of seizure activity (Heinemann et al., 1986; Vezzani et al., 1988). Glial signaling induced by low [Ca\(^{2+}\)]\(_{o}\) could therefore be involved in this association between low [Ca\(^{2+}\)]\(_{o}\) and seizures. Of interest, intractable epilepsy has been associated with alterations in glial gap junction coupling (Lee et al., 1995).

Astrocytic signaling at the gliovascular interface

Astrocytic Ca\(^{2+}\) signaling is expressed at the gliovascular interface (Simard et al., 2003). In vivo studies have identified the astrocytic end-foot processes enveloping the vessel wall as the center for purinergic signaling and Ca\(^{2+}\) wave propagation (Simard et al., 2003). These studies suggested functionality to the proteins Cx43, P2Y2 and P2Y4 expressed in a polarized fashion at the perivascular astrocytic endfeet. Multiphoton imaging of freshly prepared rat brain slices loaded with Fluo-4/AM revealed that ATP mobilized cytosolic Ca\(^{2+}\) in astrocytic end feet, whereas electrical stimulation triggered Ca\(^{2+}\) waves propagating along the vessel wall (Simard et al., 2003; Fig. 2).

The rSlo Ca\(^{2+}\)-activated K\(^{+}\) channels are also distributed in rat astrocyte perivascular endfeet and thus it is proposed that these channels may play a role in modulation of cerebral blood flow (Price et al., 2002). The role of calcium signaling in astrocytic functions related to the BBB, including water homeostasis, blood flow regulation and metabolic trafficking remain to be further characterized.

Gap junctions

In brain, astrocytes are coupled extensively by gap junctions. Gap junctions are highly conductive membrane channels that mediate metabolic coupling between glial cells by connecting their interiors. Gap junctions provide a major nonsynaptic means of communication between cortical cell types (Nadarajah et al., 1996). They are permeable to ions and to molecules less than Mr 1000 (Bennett et al., 1991) and allow direct transfer of intracellular messengers such as Ca\(^{2+}\) and IP\(_3\), between interconnected cells. Connexin 32 is expressed by oligodendrocytes and connexin 43 and 30 are expressed by astrocytes (Nadarajah et al., 1996; Nagy et al., 1999). Heterologous junctions have been identified between neurons and astrocytic processes.

Both the permeability and expression of gap junctions are tightly regulated (Giaume and McCarthy, 1996). During ischemic conditions, gap junctions remain open allowing free trafficking of secondary messengers through the cells (Cotrina et al., 1998a). Gap junctions are also required for the propagation of spreading depression (Nedergaard et al., 1995). Effective reduction of infarction volume by gap junction blockade in a rodent model of stroke has been demonstrated (Rawnduzy et al., 1997). In cultured cerebral astrocytes and in a brain slice model, gap junctional coupling is modulated by [K\(^{+}\)]\(_{o}\), elevations (Enkvist and McCarthy, 1994; Nagy and Li, 2000).

SECTION III: SIGNALING IN RELATION TO CELL VOLUME

Astrocytic signaling in relation to cell volume regulation and water homeostasis

Astrocytes are highly sensitive to changes in extracellular osmolality and are capable of exhibiting prominent swelling. Decreased external osmolarity induces transient rapid swelling in glial cells, followed by a corrective process leading to cell volume recovery (Pasantes-Morales, 1996; Pasantes-Morales et al., 2000, 2002). This is an active process accomplished by the extrusion via leak pathways, of intracellular osmoles, mainly K\(^{+}\) and Cl\(^{-}\), and organic molecules such as pyroles, and organic amines (Pasantes-Morales, 2002). Studies in cultured astrocytes document the efflux of taurine, GABA, glutamate and glycine in response to hyposmotic stimuli (Passantes-Morales, 1993). The signaling mechanisms triggered by cellular volume alterations appear to be various, including swell-activated channels (Pasantes-Morales et al., 2002; Mizuno et al., 2003). Volume-regulated anion channels (VRACs) tightly regulate cell volume homeostasis, and under pathological conditions contribute to neuronal damage via excitatory amino acid release. For instance, persistent swelling induced by lactacidosis, in glial cells appears to be sec-
ondary to the inhibition of volume-sensitive anion channels (Nabekura et al., 2003).

Acute hyponatremia appears to act as one of the secondary insults following severe brain trauma and is associated with swelling of the perivascular astrocytic endfoot processes where down-regulation of AQP4, has also been observed (Ke et al., 2002).

**Taurine and astrocytes**

Taurine is an abundant, free, sulfur-containing, β-amino acid that exerts an osmoregulatory effect in the brain (Tuz et al., 2001). Taurine is predominantly concentrated in glial cells in the supraoptic nucleus (Decavel and Hatton, 1995). This glial osmoregulatory control may be a complement to that exerted by central and peripheral osmoreceptors regulating neurosecretion in response to changes in plasma osmolarity (Oliet, 2002). The release of taurine from astrocytes during K⁺-evoked swelling and depolarization suggest that taurine participates in the mechanisms responsible for volume regulation (Pasantes-Morales and Schousboe, 1989). In primary astrocyte cultures prepared from rat cerebral cortices, a volume-dependent anion channel permeable to taurine and under the control of tyrosine kinase(s) has been described (Mongin et al., 1999b). Another such channel, permeable to both taurine and aspartate, though not regulated by tyrosine phosphorylation with regards to volume-dependent regulation, has also been reported (Mongin et al., 1999a). Based upon the measurement of quantitative differences in Ca²⁺/calmodulin sensitivity of high K⁺-induced and hypoosmotic medium-induced cell swelling, volume-dependent taurine release requires minimal basal [Ca²⁺], and involves calmodulin-independent step(s) (Bender et al., 1992). Recently, the delayed efflux of both taurine and glutamate activated at very low osmolalities has been described in conjunction with a gradual osmolarity decrease (GOD) in C6 glioma cells. The very early activation of a Cl⁻ current in this same experiment supports the concept of differing permeability pathways between amino acid and Cl⁻ efflux during GOD (Ordaz et al., 2004).

**Aquaporin-4 (AQP4)**

Aquaporins have been extensively reviewed in other chapters. Here we will briefly address the cellular distribution and presumed functionality of the tetrameric water channel AQP4 with respect to water homeostasis. Osmotic regulation in the brain involves movement of water through AQP4 membrane channels. AQP4 is anchored at the perivascular and subpial membranes by its carboxyl terminus to α-syntrophin, an adaptor protein associated with dystrophin (Amiry-Moghaddam et al., 2002). An α-syntrophin-dependent pool of AQP4 in astroglial endfeet confers bidirectional water flow between blood and brain (Amiry-Moghaddam et al., 2002). Syntrophin-null transgenic mice had mislocalized AQP4 in astroglial endfeet surrounding blood vessels and in the glia limitans associated with a delay in the onset of brain edema following IP injection of distilled water and AVP (Vajda et al., 2002). AQP4 is strongly expressed in astrocytic endfeet abutting the abluminal membrane area of cerebral capillaries (Nielsen et al., 1997; Simard et al., 2003; Furman et al., 2003), suggesting that water transport between the systemic circulation and the brain to a large extent is regulated by water permeability across astrocytic membranes (Amiry-Moghaddam et al., 2002). Moving in parallel with K⁺, water must be siphoned into blood and CSF during periods of high neuronal activity. Pools of AQP4 along the astrocytic endfeet plastering blood vessels and subjacent to the pia matter may mediate activity-dependent water fluxes required to sustain ion and volume homeostasis at central synapses (Amiry-Moghaddam and Ottersen, 2003). As water flux through AQP4 is bidirectional and driven solely by osmotic gradients, perivascular AQP4 may have negative effects in pathophysiological conditions that involve water accumulation in the brain (Amiry-Moghaddam and Ottersen, 2003). Experimental acute hyponatremia induces significant swelling of perivascular astrocytic foot processes in rats with traumatic brain injuries (Ke et al., 2002). In conjunction, knockout of AQP4 expression in mice reduced the extent of posts ischemic brain edema and glial swelling induced by hypo-osmotic stress (Manley et al., 2000). The impediment of the efflux of water in the post-ischemic phase can be attributed to the loss of AQP4 from the perivascular membrane (Amiry-Moghaddam et al., 2002). Integrity of the perivascular endfeet is also required to handle excessive amounts of K⁺ that are generated during seizures. Mice with α-Syn deletion and associated mislocalization of AQP4 in the brain have enhanced severity of hypothermia-induced seizures associated with delayed K⁺ clearance (Amiry-Moghaddam et al., 2003).

The osmosensitive organs of the brain show non-polarized distribution of glial AQP4 (Nielsen et al., 1997). The supraoptic nuclei and the circumventricular organs contain glial lamellae with uniformly high AQP4 expression along their plasma membranes. These glial lamellae also express high levels of taurine, an amino acid that acts as an endogenous ligand for glycine receptors (Hussy et al., 1997; Deleuze et al., 1998). Hypoosmotic stress causes a release of taurine onto glycine receptors of osmosensitive neurons (Hussy et al., 2000). In the renal collecting ducts, activation of these glycine receptors causes reduced secretion of antidiuretic hormone, which leads to reduced water retention in the kidney. It remains to be demonstrated, that AQP4-bearing glial lamellae have a similar function, acting as a transducer in the osmosensory response (Hussy et al., 2000) across the plasma membrane.

Translocation of molecules of water can also be associated with substrate transport, independently of an osmotic gradient as with the glutamate transporter EAAT1 (MacAulay et al., 2001; Loo et al., 2002). The role of cotransporters in water transport in the brain is discussed in detail in a separate chapter.

**Nitric oxide (NO)**

NO is considered both an intercellular and intracellular messenger and is formed in the CNS. NO is produced from the terminal guanidino nitrogen atom of L-arginine following catalysis by the enzyme NO synthase (NOS).
A constitutive form of NOS is activated by increases in \([\text{Ca}^{2+}]\), levels (Bredt and Snyder, 1990). In astrocytes from the CNS, the expression of NOSs (Vincent, 1994) can be induced by the activation of PKC (Simmons and Murphy, 1994) and NO-related nitrite production occurs rapidly in a \([\text{Ca}^{2+}]\)-dependent manner suggesting a mechanism by which these cells are involved in cerebral blood flow regulation and neurovascular coupling (Weickert et al., 1997). Lipopolysaccharides have been shown to induce a \([\text{Ca}^{2+}]\)-independent NOS in astrocyte cultures (Simmons and Murphy, 1992) and treatment of such culture with dexamethasone blocks, in a dose-related fashion, the bacterial lipopolysaccharide-induced release of nitrite (Molina-Holgado et al., 1995). Interestingly, Ang II decreases, by route of the PKC pathway, the expression of inducible NOS produced by bacterial endotoxins or cytokines in cultured astroglia (Kopinsky and Sumners, 2000). As a defense mechanism against excess NO, astrocytes, but not neurons, have been demonstrated to up-regulate the synthesis of glutathione, an antioxidant in the brain (Gegg et al., 2003).

NO has been shown to react with the superoxide anion (\(O_2^-\)) forming a reactive product, peroxynitrite (ONOO\(^-\)), which up-regulates VRAC activity via a src tyrosine kinase-dependent mechanism (Haskew et al., 2002). ONOO\(^-\) formed under pathological conditions such as cerebral ischemia has also been shown to potentiate the release of the excitatory amino acid, \(\alpha-[\text{H}]\)aspartate, from astrocyte cultures exposed to limited or marked swelling via intracellular signaling mechanisms involving tyrosine kinases (Kimelberg, 2004).

Several recent publications suggest that astrocytes are a necessary intermediary in functional hyperemia, but the role of NO production from astrocytes remains to be established.

**Glucose and glycogen**

Astrocytes are capable of complete glycolysis and oxidation of glucose. The 45 kDa form of the glucose transporter 1 is localized in oligodendrocytes and astrocytes in the rat brain (Yu and Ding, 1998). Astrocytic endfeet, enriched in glucose transporters, cover virtually all capillaries in the brain (Magistretti et al., 1999). Glucose is the main energy substrate for the brain and yields substantial amounts of water when broken down through the oxidative pathway. Astrocytes may convert all, or some of the glucose that enter their vascular endfeet to lactate before uptake by neurons, but the topic remains controversial 5 years after it was proposed (Magistretti and Pellerin, 2000).

Brain glycogen is localized almost exclusively to glia, where it undergoes continuous utilization and resynthesis (Swanson, 1992). Hydrolysis of glycogen is enhanced by an increase in \([\text{K}^+]_e\) suggesting that usage of glycogen stores are regulated not only by metabolic demands, but also by cellular activation (see below) (Hertz and Peng, 1992).

**SECTION IV: IONIC HOMEOSTASIS IN ASTROCYTES**

Astrocytes function as a buffer of extracellular \(\text{K}^+\)

Glial cells are essential for the maintenance of extracellular potassium ([K\(^+]_e\)] at a level compatible with continued neuronal function (Clausen, 1992; Ballanyi, 1995). Astrocytes are connected by an extensive network of gap junctions (Cotrina et al., 1998b) which are permeable to \(\text{K}^+\). Thus, astrocytes form a syncytium for rapid redistribution of \(\text{K}^+\) from areas with high neuronal activity. Astrocytic involvement in redistribution of \(\text{K}^+\) from the perineuronal to perivascular areas is primarily mediated by inwardly rectifying \(\text{K}^+\) channels (Sontheimer, 1992). The \(K_1\) channels are expressed in astrocytes surrounding both synapses and blood vessels in the brain (Higashi et al., 2001). Recently, the demonstration of \(rSlo\) \([\text{Ca}^{2+}]\)-activated \(\text{K}^+\) channels (\(rSlo\ \text{K}_{\text{ca}}\)) in rat astrocyte perivascular endfeet (Price et al., 2002) has suggested that this channel may participate in spatial \(\text{K}^+\) buffering. The \(rSlo\ \text{K}_{\text{ca}}\) channels are sensitive to membrane depolarization and \([\text{Ca}^{2+}]\) (Price et al., 2002). Other \(K_1\) channels, such as the \(K_{\text{v1.5}}\) channels, have also been localized to astrocytic endfeet surrounding blood vessels of the hippocampus (Roy et al., 1996).

Several distinct subtypes of astrocytes, based upon the expression of \(K_1\) channels, have been described. For example, the existence of two distinct astrocyte types has been demonstrated in freshly isolated astrocytes (Zhou and Kimelberg, 2000). One type was characterized by fast \(\text{K}^+\) uptake, and expressed relatively symmetric \(\text{K}^+\) current patterns, comprising outward \(\text{K}^+\) currents (\(I_{\text{kout}}, I_{\text{kal}}\)), and abundant inward \(\text{K}^+\) currents (\(I_{\text{kin}}\)). A second type of astrocytes was characterized by an outwardly rectifying \(\text{K}^+\) channel current (Zhou and Kimelberg, 2000).

Other mechanisms for \(\text{K}^+\) uptake in astrocytes contribute to extracellular \(\text{K}^+\) homeostasis. For instance, the stimulation of ouabain-inhibited exchange between \(\text{K}^+\) and \(\text{Na}^+\), catalyzed by the \(\text{Na}^+, \text{K}^+\)-ATPase activity. The affinity of the \(\text{K}^+\)-accumulating ATPase in astrocytes is low enough that its activity is stimulated by elevated \(\text{K}^+\) concentrations (Grisar et al., 1979; Mercado and Hernandez, 1992; Hajek et al., 1996). Active uptake of \(\text{K}^+\) into astrocytes may also be mediated by the cotransport of \(\text{Na}^+\) and \(\text{Cl}^-\), which is inhibited by the diuretics furosemide and ethacrynic acid, and by bumetanide. This cotransporter is active in astrocytes where it is stimulated by elevated \([\text{K}^+]_e\) (Tas et al., 1987; Hertz et al., 2000). Of note, spatial buffering also combines with an approximately equimolar KCl transport across glial membranes (Dietzel et al., 1989).

**K\(^+\) and water homeostasis are intimately linked**

Glial cells, via the detection of osmotic changes, are capable of initiating a CNS osmoregulatory process. Glial uptake of excess \(\text{K}^+\) causes a reduction in extracellular osmolarity (Dietzel et al., 1980, 1989). Since \(\text{K}^+\) channels do not admit water to any significant extent, the accompa-
nlying water flux must be mediated through a distinct channel (Nagelhus et al., 1999). Immunogold evidence suggests that coupling of K⁺ siphoning and water transport in rat Müller cells is mediated by a coenrichment of Kir4.1 channel and the water channel AQP4 in specific membrane domains (Nagelhus, 1999). Astrocytes respond to decreases in external osmolarity by rapid swelling, followed by a corrective process leading to cell volume recovery, usually referred to as regulatory volume decrease (RVD; Pasantes-Morales et al., 1993, 2002; Strange et al., 1996). RVD involves the efflux of metabolites and activation of swelling-activated Cl⁻ currents and K⁺ currents (Kimelberg, 1995; Darby et al., 2003). Cellular volume changes and whole cell membrane currents appear to be activated during GOD in C6 glioma cells (Ordzaz et al., 2004). Under these conditions, an outwardly rectifying Cl⁻ current sensitive to niflumic acid and to benzoic acid and two different outwardly rectifying K⁺ currents which are activated by Ca²⁺ have been described (Ordzaz et al., 2004). The type of K⁺ channel involved in the cellular regulatory response to small and large changes in volume appear to differ (Ordzaz et al., 2004).

Thus, astrocytes are capable of rapid adapting to changes in external osmolarity. It is interesting to note that receptor stimulated Ca²⁺-signaling is associated with minor transient increases in astrocytic cell volume. Since RVD is associated with release of several neuroactive compounds, including glutamate and aspartate, it is tempting to postulate that dynamic changes in astrocytic cell volume may be intimately linked to Ca²⁺ signaling events and to astrocytic modulation of synaptic activity.

Sodium ion homeostasis

Astrocytes are not electrically excitable, in part due to their relatively sparse expression of voltage-gated Na⁺ channels (Fig. 3). Voltage-gated Na⁺ channels in glia allow Na⁺ influx which fuel the glial Na⁺/K⁺-ATPase pump, thereby facilitating and possibly modulating K⁺ uptake from the extracellular space (Sontheimer et al., 1996). Na⁺/K⁺-ATPase pump extrudes Na⁺ ions in exchange for extracellular K⁺ and generates an inwardly directed gradient for Na⁺ ions, which in turn energizes other secondary ion transporters (e.g. Na⁺-Ca²⁺ exchange; Rose et al., 1998). The isoforms of Na⁺,K⁺-ATPase in astrocytes have been investigated. Studies of purified cerebral cortex astrocytes from rat and mouse in culture have failed to identify the β subunit of this enzyme (Peng et al., 1998). In fact, conditions that have been shown to increase Na⁺,K⁺-ATPase activity in astrocyte cultures such as dibutyryl cAMP, high extracellular K⁺, and glutamate did not induce the expression of β subunits (Peng et al., 1998). However, astrocytes in vivo and in co-culture with neurons express Na⁺, K⁺-ATPase isofrom α2 and β2 (Peng et al., 1997) suggesting the need for cellular interaction to alter ion transporter phenotype. These same isoforms have been identified in mouse retina Müller glia (Wetzel et al., 1999).

Chloride ion homeostasis

The intracellular chloride concentration in astrocytes approximates 20–40 mM (Waltz, 1995). Several chloride channels have been described in astrocytes (Sontheimer, 1992; Janolen, 1993; Verkhratsky and Steinhauser, 2000; Fig. 3). The expression of certain Cl⁻ currents has been ascribed to the presence of intracellular ATP (Fritsch and Edelman, 1996; Strange et al., 1996). Anion currents observed include voltage-gated or volume-activated Cl⁻ conductances (Bevin et al., 1985). Hyperpolarization-activated anion currents have been ascribed to the Cl⁻ channel termed CIC-2 (Arreola et al., 1996; Fritsch and Edelman, 1996) which has been cloned in rat brain (Thieman et al., 1992) and a Cl⁻ channel with two conductance levels, 3 and 6pS, has also been reported (Nobile et al., 2000). Hypoosmotic swelling of cultured astrocytes has been shown to evoke an outwardly rectifying anion current regulated by tyrosine and MAP kinase (Crepel et al., 1998; Ordzaz et al., 2004). An inwardly rectifying Cl⁻ current in cultured rat astrocytes has also been confirmed (Ferroni et al., 1995, 1997). Makara et al., 2001 have further demonstrated that an inwardly rectifying Cl⁻ current, in rat cortical astrocytes, is augmented by extracellular acidosis and reduced by alkalosis. The amplitude of this current has shown pronounced sensitivity to changes in extracellular pH within the physiological range of 6.4−7.9, using the whole-cell patch-clamp technique (Makara et al., 2001). Interestingly, the study of a cloned human brain glutamate transporter (EAAT1) has revealed the rapid activation of a Cl⁻ channel following glutamate permeation into Xenopus oocytes (Wadike and Kavanaugh, 1998).

It has also been demonstrated that Cl⁻ may be transported into the astrocyte by a Na⁺-K⁺-2Cl⁻ cotransporter (Waller et al., 1994; Waltz, 1995) and extruded via the Cl⁻/HCO₃⁻ anion exchange system coupled to pH regulation as described by Kimelberg, 1981.

Hydrogen ion homeostasis

Several lines of evidence suggest that astrocytes participate in the regulation of extracellular pH (Jendelova and Sykova, 1991; Rose and Deitmer, 1994) and possess carbonic anhydrase, an isoenzyme that participates in pH regulation, ion transport, and water and electrolyte balance (Maren, 1988; Tashian, 1989; Ghandour et al., 2000). Hydrogen ions are not passively distributed across the cell membranes but are actively extruded to maintain an intracellular pH close to 7.0 in brain cells in vivo (Chelser, 1990; Fig. 3). Most of the H⁺ produced during energy metabolism is rapidly buffered by proteins and other ampholytes, and the concentration of free H⁺ ions in the cytoplasm only constitute a minute fraction of the total acid load (Clausen, 1992). The clearance of H⁺ from the cytoplasm and into the extracellular space is mediated in astrocytes by several transporters such as the Na⁺-H⁺-cotransporter, Na⁺/H⁺ exchanger and either Na⁺-dependent or Na⁺-independent Cl⁻/HCO₃⁻ antiporters (Schlüter and Dömer, 1992; Clausen, 1992; Deitmer and Rose, 1996; Brookes, 1997).
Bicarbonate homeostasis

Astrocytes show a pronounced intracellular alkalinization after neuronal excitation (Hertz et al., 1992). The depolarization of astrocytes, such as that occurring during the elevation of $[K^+]_o$, is a prerequisite to the development of the intracellular alkalinization (Boyarski et al., 1989; Chelsler, 1990). A receptor-activated mechanism for extracellular alkalinisation has also been established (Kaila et al., 1992). This signaling pathway triggers an efflux of bicarbonate ions through activated GABA A channels, which are relatively permeable to bicarbonate ions in cultured astrocytes (Kaila et al., 1992; Fig. 3).

Of note, the impact of cytosolic alkalinization during $Ca^{2+}$ oscillations on the enzymatic activity of astrocytes has not yet been analyzed. It is possible that transient episodes of alkalinization, which are associated with receptor-mediated signaling have profound effects upon astrocytic metabolism.

Overview

It is clear that astrocytes are highly complex cells that respond to a variety of external stimulations. The multitask housekeeping functions of astrocytes require that they both sense and response to many signals, including changes in energy supply, neuronal activity, extracellular ion concentrations and osmolarity. As discussed in this chapter, astrocytes have receptors for most neuroactive compounds, including neurotransmitters and hormones. In general, astrocytes respond to external stimulation by activation of $Ca^{2+}$ signaling. Increases in cytosolic $Ca^{2+}$ in astrocytes are generated by the release of $Ca^{2+}$ from intracellular stores and do normally not require an influx of $Ca^{2+}$ from the interstitial space. $Ca^{2+}$ signaling events are expressed as either $Ca^{2+}$ oscillations or as propagating $Ca^{2+}$ waves. $Ca^{2+}$ oscillations are readily evoked by agonist exposure and often continue following washout. In contrast, $Ca^{2+}$ waves are only triggered by intense electrical or mechanical stimulation and rarely observed in response to agonist exposure (Venance et al., 1997).

As discussed above, astrocytic $Ca^{2+}$ signaling is also evoked by changes in the extracellular environment. For example, lowering of extracellular $Ca^{2+}$, hyposmotic conditions, local application of $K^+$, or mechanical stress are all potent inducers of astrocytic $Ca^{2+}$ signaling (Zanotti and Charles, 1997; Cotrina and Nedergaard, 1998c). Thus, $Ca^{2+}$ signaling appears to be a general response of astrocytes to multiple types of stimulations. It is therefore surprising that the functional significance of astrocytic $Ca^{2+}$ signaling remains largely unknown. It is for example not known whether astrocytic $Ca^{2+}$ signaling regulates glucose transport, potassium buffering, or water homeostasis.

In intact brain tissue, astrocytic $Ca^{2+}$ signaling is strongly expressed at the gliovascular interface, suggesting a role in modulation of blood flow. A recent publication provided the first evidence that astrocytes may be active players in local activity-induced increases in blood flow. Tight spatial and temporal coupling of neuronal activity with blood-borne energy substrate delivery is a well-established hallmark of brain function and referred to as functional hyperemia. Zonta et al. (2003a,b) described a pathway by which intense electrical firing stimulated $Ca^{2+}$ signaling in cortical astrocytes by activation of mGluR. $Ca^{2+}$ increases in perivascular endfeet were correlated spatially and temporally with arteriolar dilation. Vasodilation was blocked by mGluR antagonists and COX inhibitors, suggesting that astrocytes by their close association with excitatory synapses are activated by glutamate spillover and in turn release a vasoactive cyclooxygenase product, which mediates activity-dependent hyperemia.

The most well studied area of astrocytic $Ca^{2+}$ signaling is its role in synaptic function (Haydon, 2001). Astrocytes are activated during both excitatory and inhibitory synaptic transmission and respond with elevations in intracellular $Ca^{2+}$ concentration. $[Ca^{2+}]_i$ oscillations and waves in astrocytes now appear to represent the glial arm of a dynamic neuronal–glial signaling process. Advances within the last year have shown that stimuli, which elevate $[Ca^{2+}]_i$, in astrocytes have the potential to modulate synaptic function. Glutamate released from astrocytes during $Ca^{2+}$ signaling events is the chief messenger in astrocyte–neuronal communication (Haydon, 2001; Nedergaard et al., 2002). For example, spontaneous astrocytic $Ca^{2+}$ oscillations drive NMDA-receptor-mediated neuronal excitation in the rat ventrobasal thalamus (Parri et al., 2001), and astrocytes can potentiate inhibitory transmission in the hippocampus through a pathway that is sensitive to kainate-receptor antagonists (Kang et al., 1998). Astrocytes can by $Ca^{2+}$-dependent release of glutamate modulate the strength of synaptic transmission and thereby function as a negative feedback mechanisms reducing excessive neuronal activity.

It is critical for future studies to expand the role of astrocytic $Ca^{2+}$ signaling from synaptic transmission and define how $Ca^{2+}$ signaling events may regulate the more supportive roles of astrocytes in ion and water homeostasis.

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