

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

M. SIMARD^{a*} AND M. NEDERGAARD^b

^aUtah Diabetes Center, 615 Arapeen Drive, Suite 100, Salt Lake City, UT 84108, USA

^bDepartment of Neurosurgery, Center for Aging, University of Rochester, 601 Elmwood Avenue, Rochester, NY 14642, USA

Abstract—Astrocytes are highly complex cells that respond to a variety of external stimulations. One of the chief functions of astrocytes is to optimize the interstitial space for synaptic transmission by tight control of water and ionic homeostasis. Several lines of work have, over the past decade, expanded the role of astrocytes and it is now clear that astrocytes are active participants in the tri-partite synapse and modulate synaptic activity in hippocampus, cortex, and hypothalamus. Thus, the emerging concept of astrocytes includes both supportive functions as well as active modulation of neuronal output.

Glutamate plays a central role in astrocytic-neuronal interactions. This excitatory amino acid is cleared from the neuronal synapses by astrocytes via glutamate transporters, and is converted into glutamine, which is released and in turn taken up by neurons. Furthermore, metabotropic glutamate receptor activation on astrocytes triggers via increases in cytosolic Ca²⁺ a variety of responses. For example, calcium-dependent glutamate release from the astrocytes modulates the activity of both excitatory and inhibitory synapses. *In vivo* studies have identified the astrocytic end-foot processes enveloping the vessel walls as the center for astrocytic Ca²⁺ signaling and it is possible that Ca²⁺ signaling events in the cellular component of the blood–brain barrier are instrumental in modulation of local blood flow as well as substrate transport.

The hormonal regulation of water and ionic homeostasis is achieved by the opposing effects of vasopressin and atrial natriuretic peptide on astroglial water and chloride uptake. In conjuncture, the brain appears to have a distinct astrocytic perivascular system, involving several potassium channels as well as aquaporin 4, a membrane water channel, which has been localized to astrocytic endfeet and mediate water fluxes within the brain.

The multitask functions of astrocytes are essential for higher brain function. One of the major challenges for future studies is to link receptor-mediated signaling events in astrocytes to their roles in metabolism, ion, and water homeostasis. © 2004 IBRO. Published by Elsevier Ltd. All rights reserved.

*Corresponding author.

E-mail address: marie.simard@hsc.utah.edu (M. Simard).

Abbreviations: AGT, angiotensinogen; Ang, angiotensin; ANP, atriopeptin/atrial natriuretic peptide; AQP4, aquaporin-4; AVP, vasopressin; BBB, blood–brain barrier; GFAP, glial fibrillary acidic protein; GOD, gradual osmolarity decrease; GR, glucocorticoid receptor; IP₃, inositol triphosphate; mGlu, metabotropic glutamate; NMDA, *N*-methyl-D-aspartate; NO, nitric oxide; NOS, nitric oxide synthase; RAS, renin–Ang system; RVD, regulatory volume decrease; VRAC, volume-regulated anion channel.

0306-4522/04/\$30.00+0.00 © 2004 IBRO. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuroscience.2004.09.053

Key words: calcium, hormones, blood–brain barrier, transporter, glutamate, gap junction.

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 endfeet 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

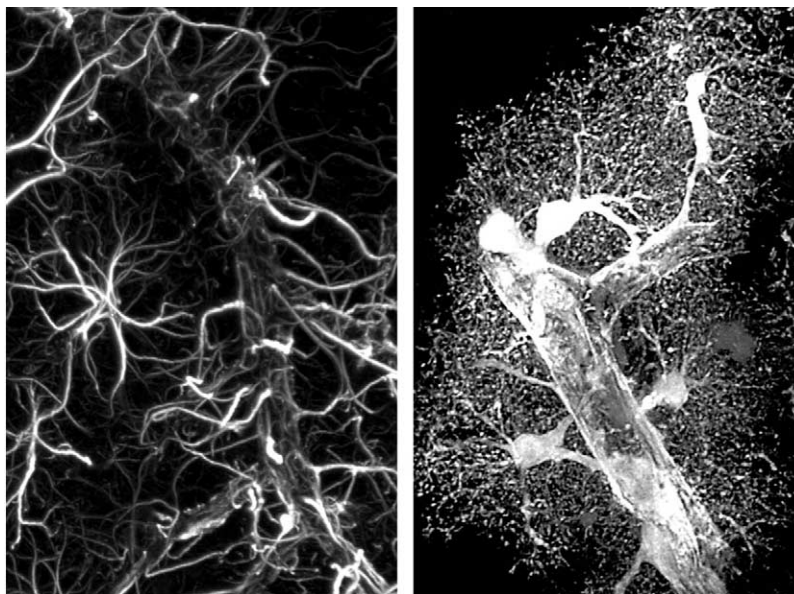


Fig. 1. Morphology of astrocytes. (A) Rat cortex stained against GFAP. GFAP is the traditional marker of astrocytes and outlines the traditional star-shaped cell types. GFAP is not present in the fine leaflet astrocytic processes and GFAP staining does only outline the major and relatively straight processes. (B) Overexpression of enhanced green fluorescence protein (eGFP) in a group of astrocytes reveals their delicate morphology *in situ*. Astrocytes are cubic or slightly elongated cells, which are characterized by their numerous fine leaflet processes. These processes are in intimate contact with neurons and cover most synapses in CNS. Astrocytes send large diameter, and often unbranched, processes to the vasculature (arrows). These vascular processes abut into endfeet, which plaster along the vessel wall. EGFP was delivered by injecting an adenovirus bearing the eGFP gene under the CMV promoter 2 days before preparing the animal for histology. Both images were acquired by serial optical sectioning by confocal microscopy.

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^+ (or two Na^+ and one H^+) in exchange for one K^+ and one OH^- (or one HCO_3^- ; Robinson and Dowd, 1997). The consequence of this stoichiometry is an increase in the Na^+ concentration within the astrocytes, accompanied by an intracellular acidification and extracellular alkalinization.

Within the astrocyte, glutamate is converted to glutamine through an ATP-requiring reaction catalyzed by the astrocyte-specific enzyme glutamine synthetase (Haller-mayer 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 $\text{Na}^+/\text{Ca}^{2+}$ -exchanger. Homocysteic acid release from astrocyte may thus contribute to the process of glia–neuron signaling.

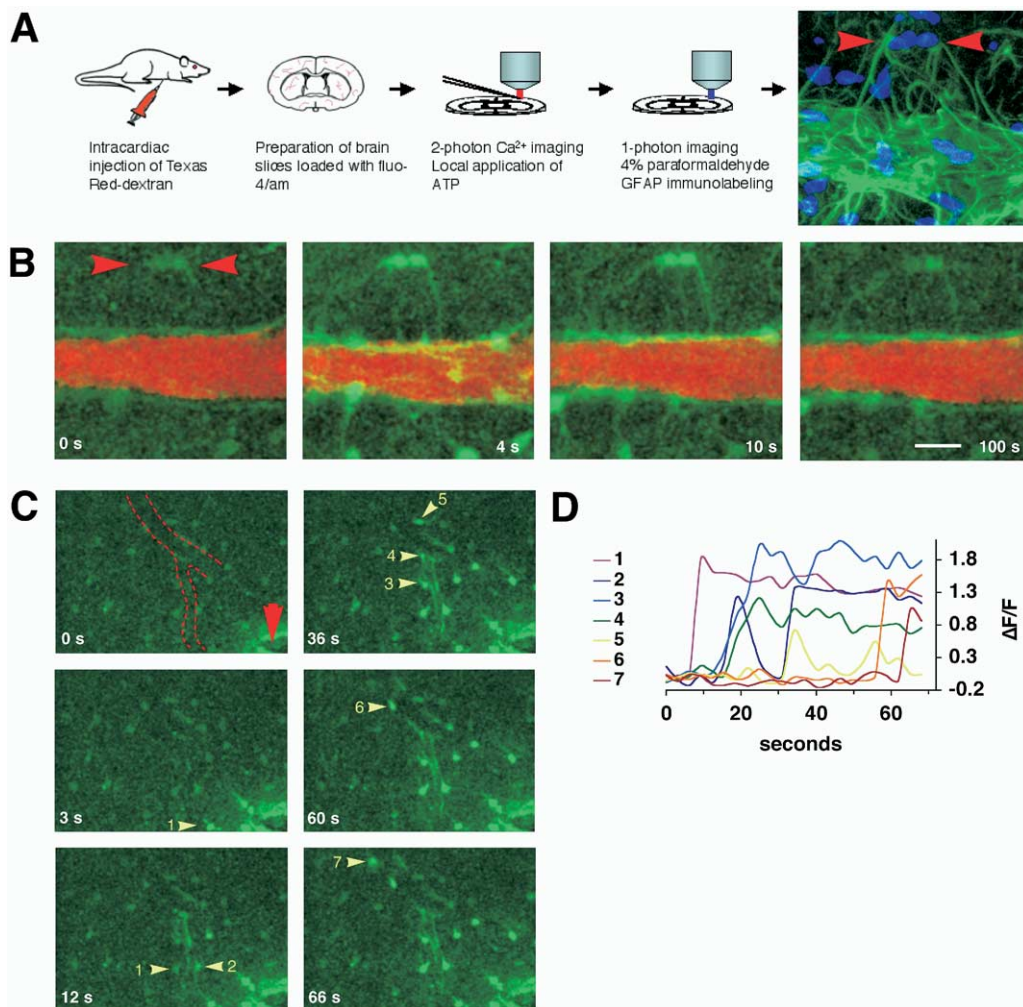


Fig. 2. (A) Preparation of freshly prepared cortical brain slices (P15) used for multiphoton imaging. Last frame visualized GFAP immunoreactivity and nuclear staining (Sytox, blue) of the same slide utilized for Ca^{2+} imaging in panel B (below). Arrowheads depict two GFAP-positive astrocytes with vascular processes that responded to ATP ($100 \mu\text{M}$) around the same vessel. The vessels were visualized by intracardiac injection of Texas Red Dextran. Arrowheads depict the GFAP-positive astrocytes in panel A, last frame. (C) Focal field stimulation (2 s pulse, 100 Hz, $100 \mu\text{A}$) evokes a Ca^{2+} wave, that propagates along the vessel wall (arrowhead, stimulation electrode: dotted line, medium-sized vessel). Astrocytes engaged in the Ca^{2+} wave is indicated by arrowheads and numbered according to the sequence they engage in Ca^{2+} signaling. (D) Relative increases in Fluo-4 signal (F/F) of the astrocytes numbered in (C). Scale bar=20 μm in (A, B); 40 μm in (C).

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, Ca^{2+} has been shown to be involved in glutamate-induced cell swelling (Koyama 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 Na^+ and glutamate (Schneider et al., 1992). The $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ cotransporters, the $\text{Na}^+\text{-K}^+\text{-ATPase}$ pump, and the 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 hypoosmotic 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

IONIC HOMEOSTASIS IN GLIA

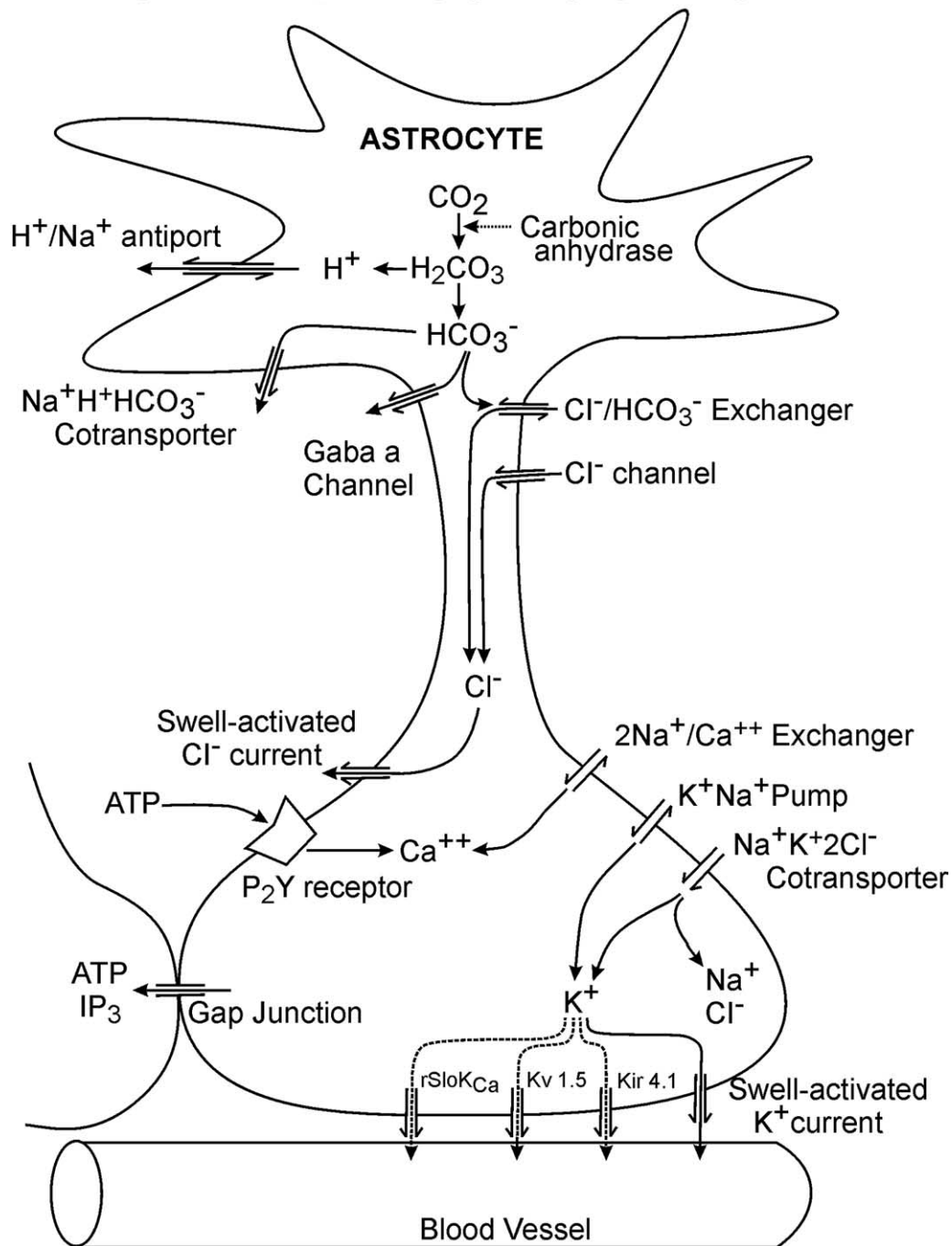


Fig. 3. Ionic homeostasis in glia. Glial cells maintain the extracellular potassium ion (K^+) concentration at levels which optimize neuronal function by actively taking up K^+ from the extracellular space via activation of inwardly rectifying K^+ channels, of K^+/Na^+ pump catalyzed by Na^+/K^+ -ATPase, and of $\text{Na}^+/\text{K}^+/\text{Cl}^-$ cotransporter. Excess intracellular K^+ is shunted to the vascular system via outwardly rectifying K^+ currents, Kir 4.1, Kv1.5, and rSloCa $^{2+}$ -activated K^+ channel (RSloK $_{\text{Ca}}$), and swell-activated K^+ currents during cellular RVD. Chloride ions (Cl^-) are transported into the astrocyte by the $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransporter and the $\text{Cl}^-/\text{HCO}_3^-$ 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 $\text{Na}^+/\text{K}^+/\text{Cl}^-$ cotransporter. Na^+ extrusion occurs via the K^+/Na^+ pump and the $2\text{Na}^+/\text{Ca}^{2+}$ exchanger. The efflux of HCO_3^- induces extracellular alkalization and occurs via activation of the GABA A channels, the $\text{Na}^+/\text{H}^+/\text{HCO}_3^-$ cotransporter, and the $\text{Cl}^-/\text{HCO}_3^-$ exchanger. Hydrogen ions (H^+) are actively extruded across the cell membrane of astrocytes via the Na^+/H^+ antiport, the $\text{Cl}^-/\text{HCO}_3^-$ anion exchanger, and the $\text{Na}^+/\text{HCO}_3^-$ cotransport. Astrocytes possess carbonic anhydrase, an isoenzyme that participates in water and electrolyte balance. The intracellular calcium ion (Ca^{2+}) concentration increases following the release of Ca^{2+} from intracellular stores induced by ATP binding to the P_2Y receptor, and following entry of Ca^{2+} through voltage-gated Ca^{2+} channels, and the $2\text{Na}^+/\text{Ca}^{2+}$ 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 (Centemeri 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 $[\text{Ca}^{2+}]_i$ (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 (Trodec 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 increas-

ing 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 (Arcuino 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 subpial 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 $[\text{Ca}^{2+}]_i$ via the V1b receptor because the potency of AVP on water uptake is the same as that on $[\text{Ca}^{2+}]_i$ (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 $[\text{K}^+]_e$ in brain by stimulation of K^+ transfer from blood to brain, due to activation of an inwardly directed $\text{Na}^+/\text{K}^+, \text{Cl}^-$

RENIN-ANGIOTENSIN SYSTEM IN GLIA

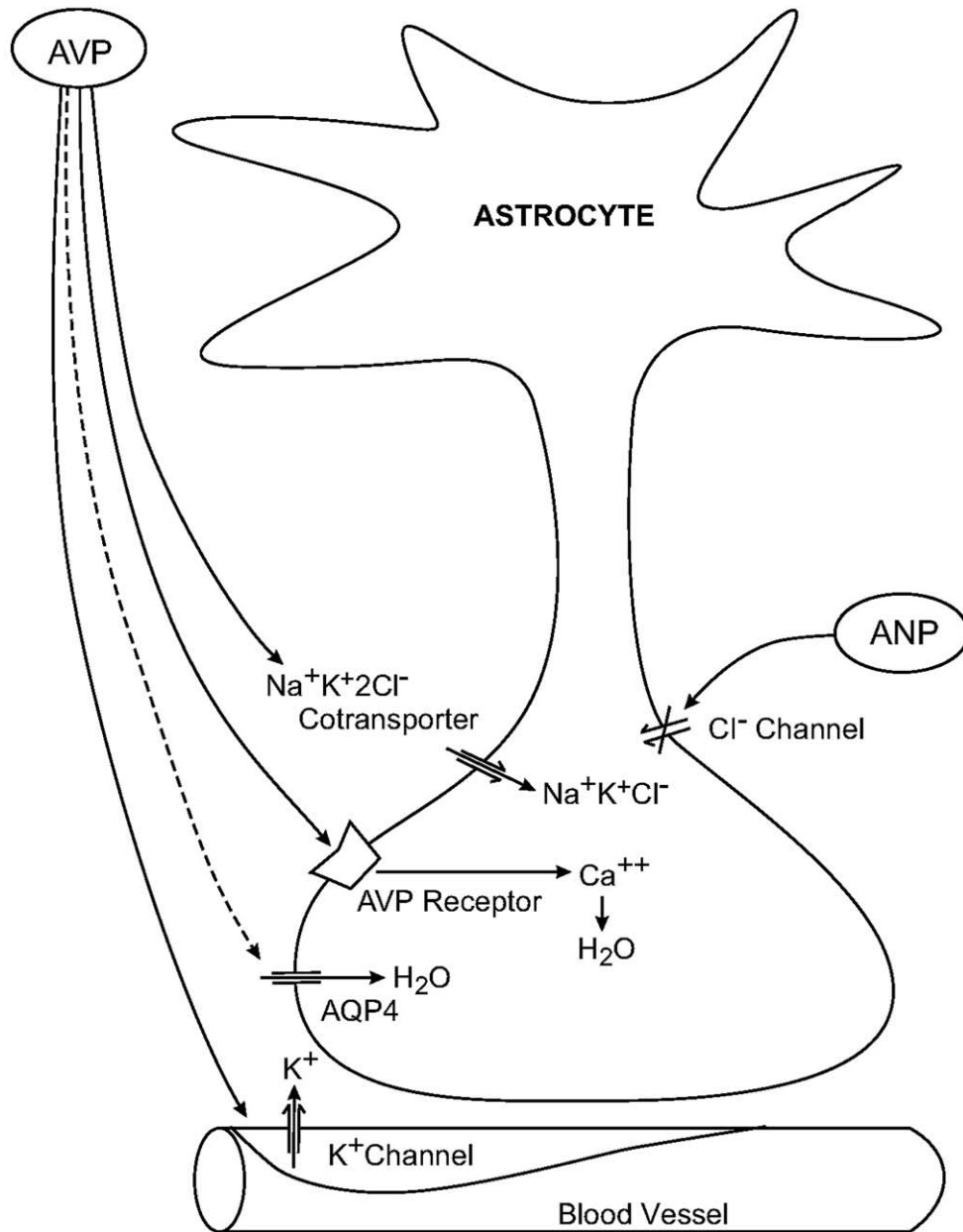


Fig. 4. The AVP–ANP system in glia. AVP has been demonstrated to induce water and cellular ionic homeostasis in astrocytes via the V1 receptor. AVP activates the $\text{Na}^+\text{K}^+\text{Cl}^-$ cotransporter thus increasing water content in astrocytes by activating the uptake of Na^+ , K^+ , and Cl^- . AVP has been shown to upregulate the expression of AQP4. ANP decreases Cl^- uptake in astrocytes.

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 $\text{Na}^+\text{K}^+2\text{Cl}^-$ 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

(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 AQP4 immunolabelling associated with the pial surface and blood vessels (Niermann et al., 2001).

ANP

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 appears to control the release of ANP stored in a distinct population of vesicles suggesting a role in glia-endothelia signaling (Kreft et al., 2004). ANP modulates unidirectional transport of sodium from blood to brain via the cGMP–PKG pathway by prohibiting amiloride-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 caused increases in AGT mRNA in presumed astrocytes in the anterior hypothala-

mus, POA, PNV, SON, suprachiasmatic nucleus, and periventricular nucleus, where as adrenalectomy 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 AGT antisense 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,

VASOPRESSIN - ATRIOPEPTIN SYSTEM IN GLIA

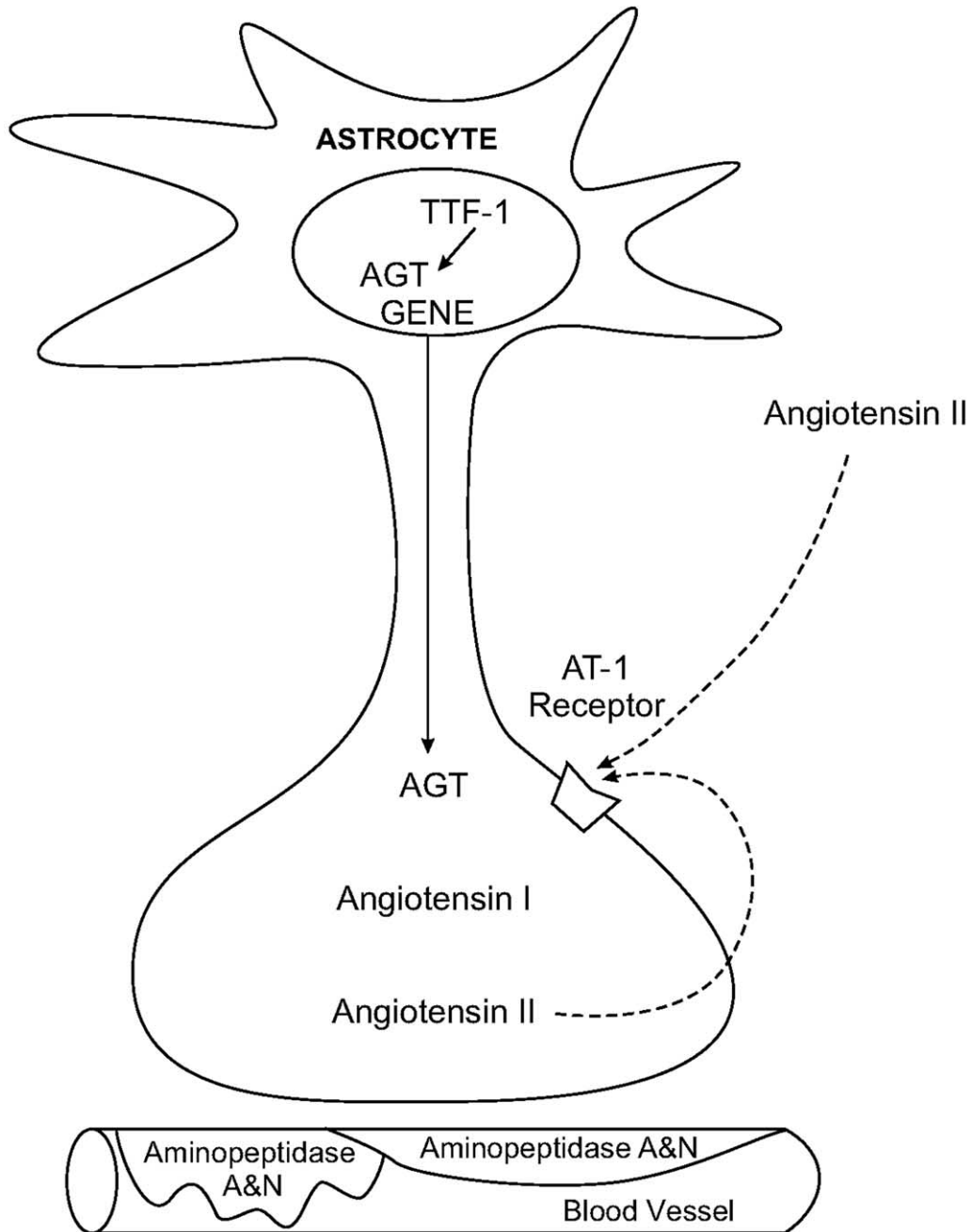


Fig. 5. The RAS in glia. Astrocytes are the primary source of ATG in the brain. TTF-1, a homeodomain-containing transcription factor, participates in the control of body fluid homeostasis by regulating AGT gene transcription. Ang I and II are detected in perivascular astrocytes which are also immunoreactive for the At-1 receptor, the binding site for Ang II. Ang II has been shown to induce Ca^{2+} signaling coupled to the expression of the At-1 receptor.

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).

Glucocorticoids

Glial 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 glial cells throughout the brain (McGimsey et al., 1991; Ahima and Harlan, 1990; Cintra et al., 1994; Joels and deKloet, 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 Ca^{2+} waves and enhance intracellular Ca^{2+} mobilization and ATP release in rat astrocytic cultures via GR signaling (Simard et al., 1999).

Glucocorticoid treatment in rats reduced glial fibrillary acidic protein (GFAP) transcription and increased glutamine synthetase transcription in hippocampal tissues (Laping et al., 1994) and in glial 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 glia may affect neighboring neural function. Steroids such as dexamethasone, hydrocortisone and progesterone have been shown to inhibit C6 astroglial cell-induced microvessel 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 glial limitans *in vivo*. In rapidly proliferating astrocytes, during the first week of secondary culture, most astrocytes exposed to dexamethasone transiently failed to express assemblies, until the time they reached confluency (Landis et al., 1991).

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

Sex hormones

Astrocytes express sex hormone receptors (Jung-Testas et al., 1992; Santagati et al., 1994). In brain astrocytes in culture, estradiol and progesterone inhibit and testosterone stimulated the Na^+ - K^+ -ATPase pump, which plays an important role in cell RVD (Fraser and Swanson, 1994). Astrocytes cultured *in vitro* have been shown to produce estradiol-17 β and express aromatase cytochrome p-450 mRNA, the enzyme that aromatizes testosterone to estradiol 17 β (Zwain et al., 1997).

SECTION II: CALCIUM SIGNALING

Ca^{2+} signaling in astrocytes

Calcium signaling plays a central role in cell activation. Ca^{2+} is a second messenger responsible for the regulation of a wide range of cellular processes (Berridge, 1994). Astrocytic Ca^{2+} signaling is expressed as oscillations in cytosolic Ca^{2+} concentrations ($[\text{Ca}^{2+}]_i$) and as slowly propagating waves of $[\text{Ca}^{2+}]_i$ increments (Smith, 1994). Astrocytes display regular oscillations when activated by stimuli including hormones.

In addition to Ca^{2+} oscillations, astrocytes also actively propagate Ca^{2+} waves. Mechanical stimulation of single astrocytes resulted in expanding waves of $[\text{Ca}^{2+}]_i$ increments, which engaged 20–100 neighboring astrocytes in culture (Charles et al., 1991; Smith, 1994). Cornell-Bell and co-workers (1990) were the first to describe that astrocytes generated propagating Ca^{2+} waves when exposed to glutamate. The discovery that astrocytes possess a mechanism for long-distance communication had a profound impact on the field and questioned the view that astrocytes are passive support cells. Later, studies showed that astrocytic Ca^{2+} waves evoked large spike-like increases in neuronal cytosolic Ca^{2+} , suggesting that astrocytes may participate directly in neurotransmission (Nedergaard, 1994; Parpura et al., 1994). It was subsequently confirmed that astrocytic Ca^{2+} signaling modulates the strength of synaptic transmission: stimulation of astrocytes led to a decrease in synaptic failure rate between pairs of synaptically coupled interneurons and CA1 pyramidal cells (Kang et al., 1998). Astrocytic Ca^{2+} waves modulate the firing frequency of both ganglion cells in dissected eyecup retinas and in hippocampal cultures (Newman and Zahs, 1998). Similarly, Schwann cells regulate neuromuscular transmission via a pathway that requires intracellular Ca^{2+} signaling (Robitaille, 1998). Together these observations identified a new signaling loop between neurons and astrocytes: astrocytes can modulate the Ca^{2+} level, and thereby the firing pattern, of neurons in their surroundings. In turn, neurons can trigger astrocytic Ca^{2+} signaling by releasing glutamate.

In rat astrocytes, *in vivo*, initiation and propagation of Ca^{2+} waves involve a sequence of intra- and intercellular steps in which purinergic receptors, gap junctions Cx43, PLC, IP_3 , and internal Ca^{2+} 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 Ca^{2+} signaling mediated by the release of ATP (Cotrina et al., 1998c). Calcium in of itself appears to have a role in triggering Ca^{2+} release from IP_3 sensitive stores in astrocytes during the generation of spontaneous $[\text{Ca}^{2+}]_i$ oscillations (Parri and Crunelli, 2003).

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

thus promote increases in $[Ca^{2+}]_i$ (Duffy and MacVicar, 1996).

Lowering extracellular Ca^{2+} triggers astrocytic Ca^{2+} signaling

Low $[Ca^{2+}]_e$ 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 $[Na^+]_e$ 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 $[Ca^{2+}]_e$ (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 $[Ca^{2+}]_e$ 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, $[Ca^{2+}]_e$ 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 $[Ca^{2+}]_e$ plays a role in the initiation of seizures. Clinically, hypocalcemia is associated with seizure activity. In an animal model, a decrease in $[Ca^{2+}]_e$ has been observed at the onset of seizure activity (Heinemann et al., 1986; Vezzani et al., 1988). Glial signaling induced by low $[Ca^{2+}]_e$ could therefore be involved in this association between low $[Ca^{2+}]_e$ 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 M_r 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 (Rawanduzy et al., 1997). In cultured cerebral astrocytes and in a brain slice model, gap junctional coupling is modulated by $[K^+]_e$ 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 osmolarity 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 osmolytes, mainly K^+ and Cl^- , and organic molecules such as pyoles, and organic amines (Pasantes-Morales, 2002). Studies in cultured astrocytes document the efflux of taurine, GABA, glutamate and glycine in response to hypoosmotic stimuli (Pasantes-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 lactic acidosis, 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 Ca^{2+} /calmodulin sensitivity of high K^+ -induced and hypoosmotic medium-induced cell swelling, volume-dependent taurine release requires minimal basal $[Ca^{2+}]_i$ and involves calmodulin-dependent step(s) (Bender et al., 1992). Recently, the delayed efflux of both taurine and glutamate activated at very low osmolarities 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 ablu-

minal 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 postischemic 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 $[Ca^{2+}]_i$ 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 Ca^{2+} -dependent manner suggesting a mechanism by which these cells are involved in cerebral blood flow regulation and neurovascular coupling (Weikert et al., 1997). Lipopolysaccharides have been shown to induce a 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 (Kopnisky 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, D - $[^3H]$ 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. Astrocyte 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 $[K^+]_e$ 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 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 K^+ . Thus, astrocytes form a syncytium for rapid redistribution of K^+ from areas with high neuronal activity. Astrocytic involvement in redistribution of K^+ from the perineuronal to perivascular areas is primarily mediated by inwardly rectifying K^+ channels (Sontheimer, 1992). The Kir 4.1 channels are expressed in astrocytes surrounding both synapses and blood vessels in the brain (Higashi et al., 2001). Recently, the demonstration of *rSlo* Ca^{2+} -activated K^+ channels (*RSlo* K_{Ca}) in rat astrocyte perivascular endfeet (Price et al., 2002) has suggested that this channel may participate in spatial K^+ buffering. The *RSlo* K_{Ca} channels are sensitive to membrane depolarization and $[Ca^{2+}]_i$ (Price et al., 2002). Other K^+ channels, such as the Kv1.5 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^+ 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 K^+ uptake, and expressed relatively symmetric K^+ current patterns, comprising outward K^+ currents (I_{Kdr} , I_{Ka}), and abundant inward K^+ currents (I_{Kin}). A second type of astrocytes was characterized by an outwardly rectifying K^+ channel current (Zhou and Kimelberg, 2000).

Other mechanisms for K^+ uptake in astrocytes contribute to extracellular K^+ homeostasis. For instance, the stimulation of ouabain-inhibited exchange between K^+ and Na^+ , catalyzed by the Na^+ , K^+ -ATPase activity. The affinity of the K^+ -accumulating ATPase in astrocytes is low enough that its activity is stimulated by elevated K^+ concentrations (Grisar et al., 1979; Mercado and Hernandez, 1992; Hajek et al., 1996). Active uptake of K^+ into astrocytes may also be mediated by the cotransport of Na^+ , and 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 $[K^+]_e$ (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). Fig. 3 depicts the various K^+ currents.

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 K^+ causes a reduction in extracellular osmolarity (Dietzel et al., 1980, 1989). Since K^+ channels do not admit water to any significant extent, the accompa-

nying 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 (Ordaz 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 Ca^{2+} have been described (Ordaz et al., 2004). The type of K^+ channel involved in the cellular regulatory response to small and large changes in volume appear to differ (Ordaz et al., 2004).

Thus, astrocytes are capable of rapid adapting to changes in external osmolarity. It is interesting to note that receptor stimulated Ca^{2+} -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 Ca^{2+} 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^+-Ca^+ 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 isoform $\alpha 2$ and $\beta 2$ (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 (Thiemann et al., 1992) and a Cl^- channel with two conductance levels, 3 and 6 pS, 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; Ordaz 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 (Wadiche 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^-/HCO_3^- 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 $Na^+-HCO_3^-$ cotransporter, Na^+/H^+ exchanger and either Na^+ -dependent or Na^+ -independent Cl^-/HCO_3^- antiporters (Schlue and Dörner, 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 $[K^+]_e$, 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 respond 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+} , hypoosmotic 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.

REFERENCES

- Ahima RS, Harlan RE (1990) Charting of type II glucocorticoid receptor-like immunoreactivity in the rat central nervous system. *Neuroscience* 39:579–604.
- Alliot F, Rutin J, Leenen PJM, Pessac B (1999a) Brain parenchyma vessels and the angiotensin system. *Brain Res* 830:101–112.
- Alliot F, Rutin J, Leeren PJM, Pessac B (1999b) Pericytes and periendothelial cells of brain parenchyma vessels co-express aminopeptidase N, aminopeptidase A, and nestin. *J Neurosci Res* 58:367–378.
- Amiry-Moghaddam M, Otsuka T, Hurn PD, Traystman RJ, Haug F-M, Froehner SC, Adams ME, Neely JD, Agre P, Ottersen OP, Bhardwaj A (2002) An α -syntrophin-dependent pool of AQP4 in astroglial endfeet confers bidirectional water flow between blood and brain. *Proc Natl Acad Sci USA* 100:2106–2111.
- Amiry-Moghaddam M, Williamson A, Palomba M, Eid T, de Lanerolle NC, Nagelhus EA, Adams ME, Froehner SC, Agre P, Ottersen OP (2003) Delayed K^+ clearance associated with aquaporin-4 mislocalization: phenotypic defects in brains of α -syntrophin-null mice. *Proc Natl Acad Sci USA* 100:13615–13620.

- Amiry-Moghaddam M, Ottersen OP (2003) The molecular basis of water transport in the brain. *Nat Rev* 4:991–1001.
- Arcuino G, Lin JH, Takano T, Liu C, Jiang L, Gao Q, Kang J, Nedergaard M (2002) Intercellular calcium signaling mediated by point-source burst release of ATP. *Proc Natl Acad Sci USA* 99:9840–9845.
- Arreola J, Park K, Melvin JE, Begenisich T (1996) Three distinct chloride channels control anion movements in rat parotid acinar cells. *J Physiol (Lond)* 490:351–362.
- Ballanyi K (1995) Modulation of glial potassium, sodium, and chloride activities by the extracellular milieu. In: *Neuroglia* (Kettenmann H, Ransom B, eds), pp 289–298. New York: Oxford University Press.
- Baltatu O, Fontes MA, Campagnole-Santos MJ, Caligiorni S, Ganten D, Santos RA, Bader M (2001a) Alterations of the renin-angiotensin system at the RVLM of transgenic rats with low brain angiotensinogen. *Am J Physiol Regul Integr Comp Physiol* 280:R428–R433.
- Baltatu O, Janssen BJ, Bricca G, Plehm R, Monti J, Ganten D, Bader M (2001b) Alterations in blood pressure and heart rate variability in transgenic rats with low brain angiotensinogen. *Hypertension* 37:408–413.
- Barr C, Rhodes P, Struthers A (1996) C-type natriuretic peptide. *Peptides* 17:1243–1251.
- Barres BA, Chun LLY, Corey DP (1990) Ion channels in vertebrate glia. *Annu Rev Neurosci* 13:441–474.
- Bender AS, Neary JT, Blicharska J, Norenberg LOB, Norenberg MD (1992) Role of calmodulin and protein kinase C in astrocytic cell volume regulation. *J Neurochem* 58:1874–1882.
- Bennett MVL, Barrio LC, Bargiello TA, Spray DC, Hertzberg EL, Saez JC (1991) Gap junctions: new tools, new answers, new questions. *Neuron* 6:305–320.
- Benz B, Grima G, Do KQ (2004) Glutamate-induced homocysteic acid release from astrocytes: possible implication in glia-neuron signaling. *Neuroscience* 124:377–386.
- Berridge MJ (1994) The biology and medicine of calcium signalling. *Mol Cell Endocrinol* 98:119–124.
- Bevin S, Chiu SY, Gray PT, Ritchie JM (1985) The presence of voltage-gated sodium, potassium and chloride channels in rat cultured astrocytes. *Proc R Soc Lond B Biol Sci* 225:299–313.
- Bezzi P, Gunderson V, Galbete JL, Seifert G, Steinhäuser C, Pilati E, Volterra A (2004) Astrocytes contain a vesicular compartment that is competent for regulated exocytosis of glutamate. *Nat Neurosci* 7:613–620.
- Blair-West JR, Burns P, Denton DA, Ferraro T, McBurnie MI, Tarjan E, Weisinger RS (1994) Thirst induced by increasing brain sodium concentration is mediated by brain angiotensin. *Brain Res* 637:335–338.
- Blankenfeld G, Kettenmann H (1992) Glutamate and GABA receptors in vertebrate glial cells. *Mol Neurobiol* 5:31–41.
- Boyarski G, Schlue WR, Ransom BR, Cragoe EJ, Boron WF (1989) Effects of ethylisopropyl amiloride on intracellular pH regulation in cultured rat astrocytes. *Acta Physiol Scand Suppl* 582:30
- Bredt DS, Snyder SH (1990) Isolation of nitric oxide synthase, a calmodulin-requiring enzyme. *Proc Natl Acad Sci USA* 87:682–685.
- Brinton RD, Gonzalez TM, Cheung WS (1994) Vasopressin-induced calcium signaling in cultured hippocampal neurons. *Brain Res* 661:274–282.
- Brookes N (1997) Intracellular pH as a regulatory signal in astrocyte metabolism. *Glia* 21:64–73.
- Brunner G, Murphy S (1993) Purinergic P_{2Y} purinergic receptors on astrocytes are directly coupled to phospholipase A₂. *Glia* 7:219–224.
- Buijs RM (1978) Intra- and extrahypothalamic vasopressin and oxytocin pathways in the rat: pathways to the limbic system, medulla oblongata and spinal cord. *Cell Tissue Res* 192:423–435.
- Centemeri C, Bolego C, Abbracchio MP, Cattabeni F, Puglisi L, Burnstock G, Nicosia S (1997) Characterization of the Ca²⁺ responses evoked by ATP and other nucleotides in mammalian brain astrocytes. *Br J Pharmacol* 121:1700–1706.
- Charles AC, Merrill JE, Dirksen ER, Sanderson MJ (1991) Intercellular signaling in glial cells: calcium waves and oscillations in response to mechanical stimulation and glutamate. *Neuron* 6:983–992.
- Chesler M (1990) The regulation and modulation of pH in the nervous system. *Prog Neurobiol* 34:401–427.
- Chen Y, Alves da Rocha MJ, Morris M (2003) Osmotic regulation of angiotensin AT1 receptor subtypes in mouse brain. *Brain Res* 965:35–44.
- Chen Y, McNeill JR, Hajek I, Hertz L (1992) Effect of vasopressin on brain swelling at the cellular level: do astrocytes exhibit a furosemide-vasopressin-sensitive mechanism for volume regulation? *Can J Physiol Pharmacol* 70:S367–S373.
- Chiu SY, Kriegler S (1994) Neurotransmitter-mediated signaling between axons and glial cells. *Glia* 11:191–200.
- Choi DW (1988) Glutamate neurotoxicity and disease of the nervous system. *Neuron* 1:623–634.
- Cintra A, Zoli M, Rosén L, Agnati LF, Okret S, Wikström AC, Gustafsson JA, Fuxe K (1994) Mapping and computer assisted morphometry and microdensitometry of glucocorticoid receptor immunoreactive neurons and glial cells in the rat central nervous system. *Neurosci* 62:893–897.
- Clausen T (1992) Potassium and sodium transport and pH regulation. *Can J Physiol Pharmacol* 70:S219–S222.
- Cornell-Bell AH, Finkbeiner SM, Cooper MS, Smith SJ (1990) Glutamate induces calcium waves in cultured astrocytes: Long-range glial signaling. *Science* 247:470–473.
- Cornell-Bell AH, Thomas PG, Caffrey JM (1992) Ca²⁺ and filopodial responses to glutamate in cultured astrocytes and neurons. *Can J Physiol Pharmacol* 70:S206–S218.
- Cotrina LM, Kang J, Lin JH-C, Bueno E, Hansen TW, He L, Liu Y, Nedergaard M (1998a) Astrocytic gap junctions remain open during ischemic conditions. *J Neurosci* 18:2520–2537.
- Cotrina ML, Lin JH-C, Alves-Rodrigues A, Liu S, Li J, Azmi-Ghadimi H, Kand J, Naus CCG, Nedergaard M (1998b) Connexins regulate calcium signaling by controlling ATP release. *Neurobiol* 95:15735–15740.
- Cotrina ML, Lin JH-C, Nedergaard M (1998c) Cytoskeletal assembly and ATP release regulate astrocytic calcium signaling. *J Neurosci* 18:8794–8804.
- Crepel V, Panenka W, Kelly ME, MacVicar BA (1998) Mitogen-activated protein and tyrosine kinases in the activation of astrocytes volume-activated chloride current. *J Neurosci* 18:1196–1206.
- Crossin KL, Tai M-H, Krushel LA, Mauro VP, Edelman GM (1997) Glucocorticoid receptor pathways are involved in the inhibition of astrocyte proliferation. *Neurobiology* 94:2687–2692.
- Danbolt NC (1994) The high-affinity uptake system for excitatory amino acids in the brain. *Prog Neurobiol* 44:377–396.
- Darby M, Kuzmiski JB, Panenka W, Feighan D, MacVicar BA (2003) ATP released from astrocytes during swelling activates chloride channels. *J Neurophysiol* 89:1870–1877.
- Davis KE, Straff DJ, Weinstein EA, Bannerman PG, Correale DM, Rothstein JD, Robinson MB (1998) Multiple signaling pathways regulate cell surface expression and activity of the excitatory amino acid carrier 1 subtype of Glu transporter in C6 glioma. *J Neurosci* 18:2475–2485.
- Davisson RL (2003) Physiological genomic analysis of the brain renin-angiotensin system. *Am J Physiol Regul Integr Comp Physiol* 285:R498–R511.
- Decavel C, Hatton GI (1995) Taurine immunoreactivity in the rat supraoptic nucleus: prominent localization in glial cells. *J Comp Neurol* 354:13–26.
- Deitmer JW, Rose CR (1996) pH regulation and proton signalling by glial cells. *Prog Neurobiol* 48:73–103.
- Deleuze C, Duvoid A, Hussy N (1998) Properties and glial origin of osmotic-dependent release of taurine from the rat supraoptic nucleus. *J Physiol (Lond)* 507:463–471.

- DePasquale M, Patlak CS, Cserr HF (1989) Brain ion and volume regulation during acute hypernatremia in Brattleboro rats. *Am J Physiol (Lond)* 256:F1059–F1066.
- de Vries GJ, Miller MA (1998) Anatomy and function of extrahypothalamic vasopressin systems in the brain. *Prog Brain Res* 119:3–20.
- Dietzel I, Heinemann U, Hofmeier G, Lux HD (1980) Transient changes in the size of the extracellular space in the sensorimotor cortex of rats in relation to stimulus-induced changes in potassium concentration. *Exp Brain Res* 40:423–432.
- Dietzel I, Heinemann U, Lux HD (1989) Relations between slow extracellular potential changes, glial potassium buffering, and electrolyte and cellular volume changes during neuronal hyperactivity in cat brain. *Glia* 2:25–44.
- Duan S, Anderson CM, Stein BA, Swanson RA (1999) Glutamate induces rapid upregulation of astrocyte glutamate transport and cell-surface expression of GLAST. *J Neurosci* 19:10193–10200.
- Duan S, Anderson CM, Keung EC, Chen Y, Swanson RA (2003) P2X7 receptor-mediated release of excitatory amino acids from astrocytes. *J Neurosci* 23:1320–1328.
- Duffy S, MacVicar BA (1996) In vitro ischemia promotes calcium influx and intracellular calcium release in hippocampal astrocytes. *J Neurosci* 16:71–81.
- Eggemont J, Trouet D, Carton I, Nilius B (2001) Cellular function and control of volume-regulated anion channels. *Cell Biochem Biophys* 35:263–274.
- Enkvist MO, McCarthy KD (1994) Astroglial gap junction communication is increased by treatment with either glutamate or high K⁺ concentration. *J Neurochem* 62:489–495.
- Ennis SR, Ren XD, Betz AL (1996) Mechanisms of sodium transport at the blood-brain barrier studied with *in situ* perfusion of rat brain. *J Neurochem* 66:756–763.
- Fam SR, Gallagher CJ, Salter MW (2000) P2Y(1) purinoceptor-mediated Ca⁽²⁺⁾ signaling and Ca⁽²⁺⁾ wave propagation in dorsal spinal cord astrocytes. *J Neurosci* 20:2800–2808.
- Ferrario CM, Brosnihan KB, Diz DI, Jaiswai N, Khosla MC, Milsted A, Tallant EA (1991) Angiotensin(1–7): a new hormone of the angiotensin system. *Hypertension* 18(Suppl III):III-126–III-133.
- Ferroni S, Marchini C, Nobile M, Rapisarda C (1997) Characterization of an inwardly rectifying chloride conductance expressed by cultured rat cortical astrocytes. *Glia* 21:217–227.
- Ferroni S, Marchini C, Schubert P, Rapisarda C (1995) Two distinct inwardly rectifying conductances are expressed in long term dibutyl-AMP treated rat cultured cortical astrocytes. *FEBS Lett* 367:319–325.
- Fitzsimons JT (1998) Angiotensin, thirst, and sodium appetite. *Physiol Rev* 78:583–670.
- Fonnum F (1984) Glutamate: a transmitter in mammalian brain. *J Neurochem* 42:1–11.
- Franke H, Krugel U, Schmidt R, Grosche J, Reichenbach A, Illes P (2001) P2 receptor-types involved in astrogliosis *in vivo*. *Br J Pharmacol* 134:1180–1189.
- Fraser CL, Swanson RA (1994) Female sex hormones inhibit volume regulation in rat brain astrocyte culture. *Am J Physiol Cell Physiol* 267:C909–C914.
- Fritsch J, Edelman A (1996) Modulation of the hyperpolarization-activated Cl⁻ current in human intestinal T84 epithelial cells by phosphorylation. *J Physiol (Lond)* 490:115–128.
- Fukui S, Fazzina G, Amorini AM, Dunbar JG, Marmor A (2003) Differential effects of atrial natriuretic peptide on the brain water and sodium after experimental cortical contusion in the rat. *J Cereb Blood Flow Metab* 23:1212–1218.
- Furman CS, Gorelick-Feldman DA, Davidson KGV, Yasumura T, Neely JD, Agre P, Rash JE (2003) Aquaporin-4 square array assembly: opposing actions of M1 and M23 isoforms. *Proc Natl Acad Sci USA* 100:13609–13614.
- Gallo V, Giovanni C, Suergiu R, Levi G (1989) Expression of excitatory amino acid receptors by cerebellar cells of type-2 astrocyte cell lineage. *J Neurochem* 52:1–9.
- Gebke E, Müller AR, Jurzak M, Gerstberger R (1998) Angiotensin II-induced calcium signalling in neurons and astrocytes of rat circumventricular organs. *Neuroscience* 85:509–520.
- Gegg ME, Beltran B, Salas-Pino S, Bolanos JP, Clark JB, Moncada S, Heales SJR (2003) Differential effect of nitric oxide on glutathione metabolism and mitochondrial function in astrocytes and neurons: implications for neuroprotection/neurodegeneration? *J Neurochem* 86:228–237.
- Ghandour MS, Parkkila A-K, Parkkila S, Waheed A, Sly WS (2000) Mitochondrial carbonic anhydrase in the nervous system: expression in neuronal and glial cells. *J Neurochem* 75:2212–2220.
- Giaume C, McCarthy KD (1996) Control of gap-junctional communication in astrocytic networks. *Trends Neurosci* 19:319–325.
- Goldman WF, Yarowsky PJ, Juhaszova M, Krueger BK, Mordecai P (1994) Sodium/calcium exchange in rat cortical astrocytes. *J Neurosci* 14:5834–5843.
- Grisar T, Frère JM, Franck G (1979) Effects of K⁺ ions on kinetic properties of the (Na₊,K₊) ATPase (EC 3.6.1.3.) of bulk isolated glia, perikarya and synaptosomes from rabbit brain cortex. *Brain Res* 165:87–103.
- Guthrie PB, Knappenberger J, Segal M, Bennett MV, Charles AC, Kater SB (1999) ATP released from astrocytes mediates glial calcium waves. *J Neurosci* 19:520–528.
- Hajek I, Subbarao KV, Hertz L (1996) Stimulation of Na⁺, K⁺-ATPase activity in astrocytes and neurons by K⁺ and/or noradrenaline. *Neurochem Int* 28:335–342.
- Hallermayer K, Harmening C, Hamprecht B (1981) Cellular localization and regulation of glutamine synthetase in primary cultures of brain cells from newborn mice. *J Neurochem* 37:43–52.
- Haskew RE, Mongin AA, Kimelberg HK (2002) Peroxynitrite enhances astrocytic volume-sensitive excitatory amino acid release via src tyrosine kinase-dependent mechanism. *J Neurochem* 82:903–912.
- Haydon PG (2001) GLIA: listening and talking to the synapse. *Nat Rev Neurosci* 2:185–193.
- Heinemann U, Konnerth A, Pumain R, Wadman WJ (1986) Extracellular calcium and potassium concentration changes in chronic epileptic brain tissue. *Adv Neurol* 44:641–661.
- Hertz L, Chen Y, Spatz M (2000) Involvement of non-neuronal brain cells in AVP-mediated regulation of water space at the cellular, organ, and whole-body level. *J Neurosci Res* 62:480–490.
- Hertz L, Peng L (1992) Energy metabolism at the cellular level of the CNS. *Can J Physiol Pharmacol* 70:S145–S157.
- Higashi K, Fujita A, Inanobe A, Tanemoto M, Doi K, Kubo T, Kurachi Y (2001) An inwardly rectifying K⁺ channel, Kir4.1, expressed in astrocytes surrounds synapses and blood vessels in brain. *Am J Physiol Cell Physiol* 281:C922–C931.
- Hosli L, Hosli E, Lefkovits M, Wagner S (1991) Electrophysiological evidence for the existence of receptors for endothelin and vasopressin on cultured astrocytes of rat spinal cord and brain stem. *Neurosci Lett* 131:193–195.
- Holthoff K, Witte OW (2000) Directed spatial potassium redistribution in the rat neocortex. *Glia* 29:288–292.
- Hussy N, Deleuze C, Desarmenien MG, Moos FC (2000) Osmotic regulation of neuronal activity: a new role for taurine and glial cells in a hypothalamic neuroendocrine structure. *Prog Neurobiol* 62:113–134.
- Hussy N, Deleuze C, Pantaloni A, Desarmenien MG, Moos F (1997) Agonist action of taurine on glycine receptors in rat supraoptic magnocellular neurons: possible role in osmoregulation. *J Physiol (Lond)* 502:609–621.
- Ibaragi M, Niwa M, Ozaki M (1989) Atrial natriuretic peptide modulates amiloride-sensitive Na⁺ transport across the blood-brain barrier. *J Neurochem* 53:1802–1806.
- Janolen T (1993) Single-channel characteristics of the large-conductance anion channel in rat cortical astrocytes in primary culture. *Glia* 9:227–237.

- Jeftinija SD, Jeftinija KV, Stefanovic G, Liu F (1996) Neuroligand-evoked calcium-dependent release of excitatory amino acids from cultured astrocytes. *J Neurochem* 66:676–684.
- Jendelova P, Sykova E (1991) Role of glia in K^+ and pH homeostasis in the neonatal rat spinal cord. *Glia* 4:56–63.
- Joels M, deKloet ER (1994) Mineralocorticoid and glucocorticoid receptors in the brain, implications for ion permeability and transmitter systems. *Prog Neurobiol* 43:1–36.
- Jordan CL (1999) Glia as mediators of steroid hormone action on the nervous system: an overview. *J Neurobiol* 40:434–445.
- Junankar PR, Kirk K (2000) Organic osmolyte channels: a comparative review. *Cell Physiol Biochem* 10:355–360.
- Jung-Testas I, Renoir M, Bugnard H, Greene GL, Baulieu E-E (1992) Demonstration of steroid hormone receptors and steroid action in primary cultures of rat glial cells. *J Steroid Biochem Mol Biol* 41:621–631.
- Juurlink BJJ, Schousboe A, Jørgensen OS, Hertz L (1981) Induction by hydrocortisone of glutamine synthetase in mouse primary astrocyte cultures. *J Neurochem* 36:136–142.
- June Son Y, Kyu Hur M, Jun Ryu B, Park SK, Damante G, Valentina D'Elia A, Costa ME, Ojeda SR, Lee BJ (2003) TTF-1, a homeodomain-containing transcription factor, participates in the control of body fluid homeostasis by regulating angiotensinogen gene transcription in the rat subfornical organ. *J Biol Chem* 278:27043–27052.
- Kaila K, Paaslasmaa P, Taira T, Voipio J (1992) pH transients due to monosynaptic activation of GABA A receptors in rat hippocampal slices. *Neuroreport* 3:105–108.
- Kandel ER (1991) Nerve cell and behavior. In: Principles of neural science (Kandel ER, Schwartz JH, Jessel TM, eds), pp 18–32. New York: Elsevier Science Publishing.
- Kang J, Jiang L, Goldman SA, Nedergaard M (1998) Astrocyte-mediated potentiation of inhibitory synaptic transmission. *Nat Neurosci* 1:683–692.
- Katay L, Latzkovits L, Fonagy A, Janka Z, Lajtha A (1998) Effects of arginine vasopressin and atriopeptin on chloride uptake in cultured astroglia. *Neurochem Res* 23:831–836.
- Ke C, Poon WS, Keung Ng H, Lai FMM, Tang NLS, Pang JCS (2002) Impact of experimental acute hyponatremia on severe traumatic brain injury in rats: influences on injuries, permeability of blood-brain barrier, ultrastructural features, and aquaporin-4 expression. *Exp Neurol* 178:194–206.
- Kimelberg HK (1981) Active accumulation and exchange transport of chloride in astroglial cells in culture. *Biochim Biophys Acta* 646:179–184.
- Kimelberg HK (1995) Brain edema. In: Neuroglia (Kettenmann H, Ransom B, eds), pp 919–935. New York: Oxford University Press.
- Kimelberg HK (2004) Increased release of excitatory amino acids by actions of ATP and peroxyntirite on volume-regulated anion channels (VRACs) in astrocytes. *Neurochem Int* 45:511–519.
- Kimelberg HK, Goderie S, Higman S, Pang S, Waniewski R (1990) Swelling-induced release of glutamate, aspartate, and taurine from astrocyte cultures. *J Neurosci* 10:1583–1591.
- Kopnisky KL, Summers C (2000) Angiotensin II-induced decrease in expression of inducible nitric oxide synthase in rat astroglial cultures: role of protein kinase C. *J Neurochem* 74:613–620.
- Koyama Y, Baba A, Iwata H (1991) L-Glutamate induced swelling of cultured astrocytes is dependent on extracellular Ca^{2+} . *Neurosci Lett* 122:210–212.
- Krebs C, Fernandes HB, Sheldon C, Raymond LA, Baimbridge KG (2003) Functional NMDA receptor subtype 2B is expressed in astrocytes after ischemia *in vivo* and anoxia *in vitro*. *J Neurosci* 23:3364–3372.
- Kreff M, Stenovec M, Rupnik M, Grilc S, Kržan M, Potokar M, Pangršič T, Haydon PG, Zorec R (2004) Properties of Ca^{2+} -dependent exocytosis in cultured astrocytes. *Glia* 46:437–445.
- Kristian T, Gido G, Siesjo BK (1996) Brain calcium metabolism in hypoglycemic coma. *J Cereb Blood Flow Metab* 13:955–961.
- Landgraf R (1992) Central release of vasopressin: stimuli, dynamics, consequences. *Prog Brain Res* 91:29–39.
- Landis DMD, Weinstein LA, Skordeles CJ (1991) Effects of dexamethasone on the differentiation of membrane structure in cultured astrocytes. *Glia* 4:335–344.
- Laping NJ, Nichols NR, Day JR, Johnson SA, Finch CE (1994) Transcriptional control of glial fibrillary acidic protein and glutamine synthetase *in vivo* shows opposite responses to corticosterone in the hippocampus. *Endocrinology* 135:1928–1933.
- Latour I, Hamid J, Beedle AM, Zamponi GW, Macvicar BA (2003) Expression of voltage-gated Ca^{2+} channel subtypes in cultured astrocytes. *Glia* 41:347–353.
- Latzkovits L, Cserr HF, Park JT, Patlak CS, Pettigrew KD, Rimanoczy A (1993) Effects of vasopressin (VP) and atriopeptin (AP) on glial cell volume measured as 3-MG space. *Am J Physiol Cell Physiol* 264:C603–C608.
- Lee SH, Magge S, Spencer DD, Sontheimer H, Cornell-Bell AH (1995) Human epileptic astrocytes exhibit increased gap junction coupling. *Glia* 15:195–202.
- Liao S-L, Chen C-J (2003) L-Glutamate decreases glucose utilization by rat cortical astrocytes. *Neurosci Lett* 348:81–84.
- Loo DT, Althoen MC, Cotman CW (1995) Differentiation of serum-free mouse embryo cells into astrocytes is accompanied by induction of glutamine synthetase activity. *J Neurosci Res* 42:184–191.
- Loo DDF, Wright EM, Zeuthen T (2002) Water pumps. *J Physiol (Lond)* 542:53–60.
- MacAulay N, Gether U, Klaerke DA, Zeuthen T (2001) Water transport by the human Na^+ -coupled glutamate cotransporter expressed in *Xenopus* oocytes. *J Physiol (Lond)* 530:367–378.
- MacAulay N, Gether U, Klaerke DA, Zeuthen T (2002) Passive water and urea permeability of human Na^+ -glutamate cotransporter expressed in *Xenopus* oocytes. *J Physiol* 542:817–828.
- MacVicar BA, Hochman D, Delay MJ, Weiss S (1991) Modulation of intracellular Ca^{++} in cultured astrocytes by influx through volume activated Ca^{++} channels. *Glia* 4:448–455.
- Magistretti PJ, Pellerin L, Rothman DL, Shulman RG (1999) Energy on demand. *Science* 283:496–497.
- Magistretti PJ, Pellerin L (2000) The astrocyte-mediated coupling between synaptic activity and energy metabolism operates through volume transmission. *Prog Brain Res* 125:229–240.
- Makara JK, Petheö GL, Tóth A, Spät A (2001) pH-sensitive inwardly rectifying chloride current in cultured rat cortical astrocytes. *Glia* 34:52–58.
- Manley GT, Fujimura M, Ma T, Noshita N, Filiz F, Bollen AW, Chan P, Verkman AS (2000) Aquaporin-4 deletion in mice reduces brain edema after acute water intoxication and ischemic stroke. *Nat Med* 6:159–163.
- Maren TH (1988) The kinetics of HCO_3^- synthesis related to fluid secretion, pH control, and CO_2 elimination. *Annu Rev Physiol* 50:695–717.
- Martinez E, Wahli W (1991) Characterization of hormone response elements. In: Nuclear hormone receptors: molecular mechanisms, cellular functions, clinical abnormalities (Parker MG, ed), pp 125–153. London: Academic Press.
- McGimsey WC, Cidlowski JA, Stumpf WE, Sar M (1991) Immunocytochemical localization of the glucocorticoid receptor in rat brain, pituitary, liver, and thymus with two new polyclonal antipeptide antibodies. *Endocrinology* 129:3064–3072.
- Mercado R, Hernandez J (1992) Regulatory role of a neurotransmitter (5-HT) on glial Na^+/K^+ -ATPase in the rat brain. *Neurochem Int* 21:119–127.
- Meyer FB (1989) Calcium, neuronal hyperexcitability and ischemic injury. *Brain Res Rev* 14:227–243.
- Mizuno A, Matsumoto N, Imai M, Suzuki M (2003) Impaired osmotic sensation in mice lacking TRPV4. *Am J Physiol Cell Physiol* 285:C96–C101.
- Molina-Holgado F, Lledo A, Guaza C (1995) Evidence for cyclooxygenase activation by nitric oxide in astrocytes. *Glia* 15:167–172.

- Mongin AA, Cai Z, Kimelberg HK (1999a) Volume-dependent taurine release from cultured astrocytes requires permissive $[Ca^{2+}]_i$ and calmodulin. *Am J Physiol Cell Physiol* 277:C823–C832.
- Mongin AA, Reddi JM, Charniga C, Kimelberg HK (1999b) $[^3H]$ taurine and $D-[^3H]$ aspartate release from astrocyte cultures are differently regulated by tyrosine kinase. *Am J Physiol Cell Physiol* 276:C1226–C1230.
- Montana V, Ni Y, Sunjara V, Hua X, Parpura V (2004) Vesicular glutamate transporter-dependent glutamate release from astrocytes. *J Neurosci* 24:2633–2642.
- Morimoto S, Cassell MD, Sigmund CD (2003) Glia- and neuron-specific expression of the renin-angiotensin system in brain alters blood pressure, water intake, and salt preference. *J Biol Chem* 277:33235–33241.
- Munir M, Correale DM, Robinson MB (2000) Substrate-induced up-regulation of $Na^{(+)}$ -dependent glutamate transport activity. *Neurochem Int* 37:147–162.
- Nabekura T, Morishima S, Cover TL, Mori S-I, Kannan H, Komune S, Okada Y (2003) Recovery from lacticidosis-induced glial cell swelling with the aid of exogenous anion channels. *Glia* 41:247–259.
- Nadarajah B, Thomaidou D, Evans WH, Parnavelas JG (1996) Gap junctions in the adult cerebral cortex: regional differences in their distribution and cellular expression of connexins. *J Comp Neurol* 376:326–342.
- Nagelhus EA, Horio Y, Inanobe A, Fujita A, Haug F-M, Nielsen S, Kurachi Y, Ottersen OP (1999) Immunogold evidence suggests that coupling of K^{+} siphoning and water transport in rat retinal Müller cells is mediated by a coenrichment of Kir4.1 in specific membrane domains. *Glia* 26:47–54.
- Nagelhus EA, Lehmann A, Ottersen OP (1996) Neuronal and glial handling of glutamate and glutamine during hypoosmotic stress: a biochemical and quantitative immunochemical analysis using the rat cerebellum as a model. *Neuroscience* 72:743–755.
- Nagy JI, Patel D, Ochalski PAY, Stelmack GL (1999) Connexin 30 in rodent, cat and human brain: selective expression in gray matter astrocytes, co-localization with connexin 43 at gap junctions and late developmental appearance. *Neuroscience* 88:447–468.
- Nagy JI, Li WE (2000) A brain slice model for in vitro analyses of astrocytic gap junction and connexin43 regulation: actions of ischemia, glutamate and elevated potassium. *Eur J Neurosci* 12:4567–4572.
- Namura S, Maeno H, Takami S, Jiang X-F, Kamichi S, Wada K, Nagata I (2002) Inhibition of glial glutamate transporter GLT-1 augments brain edema after transient focal cerebral ischemia in mice. *Neurosci Lett* 342:117–120.
- Nedergaard M (1994) Direct signaling from astrocytes to neurons in cultures of mammalian brain cells. *Science* 263:1768–1771.
- Nedergaard M, Cooper AJL, Goldman SA (1995) Gap junctions are required for the propagation of spreading depression. *J Neurobiol* 28:433–444.
- Nedergaard M, Takano T, Hansen AJ (2002) Beyond the role of glutamate as a neurotransmitter. *Nat Rev Neurosci* 3:748–755.
- Newman EA, Zahs KR (1998) Modulation of neuronal activity by glial cells in the retina. *J Neurosci* 18:4022–4028.
- Nielsen S, Nagelhus EA, Amiry-Moghaddam M, Nagelhus EA, Amiry-Moghaddam M, Bourque C, Agre P, Ottersen OP (1997) Specialized membrane domains for water transport in glial cells: high-resolution immunogold cytochemistry of aquaporin-4 in rat brain. *J Neurosci* 17:171–180.
- Niermann H, Amiry-Moghaddam M, Holthoff K, Witte OW, Ottersen OP (2001) A novel role of vasopressin in the brain: modulation of activity-dependent water flux in the neocortex. *J Neurosci* 21:3045–3051.
- Nobile M, Pusch M, Rapisarda C, Ferroni S (2000) Single-channel analysis of a Cl⁻-like chloride conductance in cultured rat cortical astrocytes. *FEBS Lett* 479:10–14.
- O'Banion MK, Young DA, Bohn MC (1994) Corticosterone-responsive mRNA in primary rat astrocytes. *Brian Res Mol Brain Res* 22:57–68.
- O'Connor ER, Kimelberg HK (1993) Role of calcium in astrocyte volume regulation and in the release of ions and amino acids. *J Neurosci* 13:2638–2650.
- Oliet SHR, Piet R, Poulain DA (2001) Control of glutamate clearance and synaptic efficacy by glial coverage of neurons. *Science* 292:923–926.
- Oliet SRH (2002) Functional consequences of morphological neuroglial changes in the magnocellular nuclei of the hypothalamus. *J Neuroendocrinol* 4:241–248.
- Ordaz B, Vaca L, Franco R, Pasantes-Morales H (2004) Volume changes and whole cell membrane currents activated during gradual osmolarity decrease in C6 glioma cells: contribution of two types of K^{+} channels. *Am J Physiol Cell Physiol* 286:C1399–C1409.
- Parpura V, Basarsky TA, Liu F, Jęftinija K, Jęftinija S, Haydon PG (1994) Glutamate-mediated astrocyte-neuron signalling. *Nature* 369:744–747.
- Parpura V, Scemes E, Spray DC (2004) Mechanisms of glutamate release from astrocytes: gap junction “hemichannels,” purinergic receptors and exocytotic release. *Neurochem Int* 45:259–264.
- Parri HR, Gould TM, Crunelli V (2001) Spontaneous astrocytic Ca^{2+} oscillations in situ drive NMDAR-mediated neuronal excitation. *Nat Neurosci* 4:803–812.
- Parri HR, Crunelli V (2003) The role of Ca^{2+} in the generation of spontaneous astrocytic Ca^{2+} oscillations. *Neuroscience* 120:979–992.
- Pasantes-Morales H (1996) Volume regulation in brain cells: cellular and molecular mechanisms. *Metab Brain Dis* 11:187–204.
- Pasantes-Morales H, Alavez S, Sanchez Olea R, Moran J (1993) Contribution of organic and inorganic osmolytes to volume regulation in rat brain cells in culture. *Neurochem Res* 18:445–452.
- Pasantes-Morales H, Cardin V, Tuz K (2000) Signaling events during swelling and regulatory volume decrease. *Neurochemistry* 25:1301–1314.
- Pasantes-Morales H, Franco R, Ochoa L, Ordaz B (2002) Osmosensitive release of neurotransmitter amino acids: relevance and mechanisms. *Neurochem Res* 27:59–65.
- Pasantes-Morales H, Schousboe A (1989) Release of taurine from astrocytes during potassium-evoked swelling. *Glia* 2:45–50.
- Peng L, Arystarkhova E, Sweadner KJ (1998) Plasticity of Na, K-ATPase isoform expression in cultures of rat astrocytes: species differences in gene expression 24:257–271.
- Peng L, Martin-Vasallo P, Sweadner KJ (1997) Isoforms of Na, K-ATPase alpha and beta subunits in the rat cerebellum and in granule cell cultures. *J Neurosci* 17:3488–3502.
- Poitry-Yamate CL, Vutskits L, Rauen T (2002) Neuronal-induced and glutamate-dependent activation of glial glutamate transport function. *J Neurochem* 82:987–997.
- Price DL, Ludwig JW, Mi H, Schwarz, Ellisman MH (2002) Distribution of rSlo Ca^{2+} -activated K^{+} channels in rat astrocyte perivascular endfeet. *Brain Res* 956:183–193.
- Queiroz G, Meyer DK, Meyer A, Starke K, von Kugelgen I (1999) A study of the mechanism of the release of ATP from rat cortical astroglial cells evoked by activation of glutamate receptors. *Neuroscience* 91:1171–1181.
- Rauen T, Taylor WR, Kuhlbrodt K, Wiessner M (1998) High-affinity glutamate transporters in the rat retina: a major role of the glial glutamate transporter GLAST-1 in transmitter clearance. *Cell Tissue Res* 291:19–31.
- Rawanduzy A, Hansen A, Hansen TW, Nedergaard M (1997) Effective reduction of infarct volume by gap junction blockade in a rodent model of stroke. *Neurosurg Focus* 2:1–5.
- Robinson IC (1983) Neurohypophysial peptides in cerebrospinal fluid. *Prog Brain Res* 60:129–145.

- Robinson MB, Dowd LA (1997) Heterogeneity and functional properties of subtypes of sodium-dependent glutamate transporters in the mammalian central nervous system. *Adv Pharmacol* 37:69–115.
- Robitaille R (1998) Modulation of synaptic efficacy and synaptic depression by glial cells at the frog neuromuscular junction. *Neuron* 21:847–855.
- Rose CR, Deitmer JW (1994) Evidence that glial cells modulate extracellular pH transients induced by neuronal activity in the leech central nervous system. *J Physiol (Lond)* 481:1–5.
- Rose CR, Waxman SG, Ransom BR (1998) Effects of glucose deprivation, chemical hypoxia, and stimulated ischemia on Na^+ homeostasis in rat spinal cord astrocytes. *J Neurosci* 18:3554–3562.
- Rossi DJ, Oshima T, Attwell D (2000) Glutamate release in severe brain ischaemia is mainly by reversed uptake. *Nature* 403:316–321.
- Rothman SM, Olney JW (1986) Glutamate and the pathophysiology of hypoxic-ischemic brain damage. *Ann Neurol* 19:105–111.
- Rothstein JD, Dykes-Hoberg M, Pardo CA, Bristol LA, Jin L, Kuncic RW, Kanai Y, Hediger MA, Wang Y, Schielke JP, Welty DF (1996) Knockout of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. *Neuron* 16:675–686.
- Roy ML, Saal D, Perney T, Sontheimer H, Waxman SG, Kaczmarek LK (1996) Manipulation of the delayed rectifier Kv 1.5 potassium channel in glial cells by antisense oligodeoxynucleotides. *Glia* 18:177–184.
- Ryan MC, Shen P-J, Gundlach A (1997) Angiotensinogen and natriuretic peptide mRNAs in rat brain: localization and differential regulation by adrenal steroids in hypothalamus. *Peptides* 18:495–505.
- Santagati S, Melcangi RC, Celotti F, Martini L, Maggi A (1994) Estrogen receptor is expressed in different types of glial cells in culture. *J Neurochem* 63:2058–2064.
- Sarfaraz D, Fraser CL (1999) Effects of arginine vasopressin on cell volume regulation in brain astrocyte in culture. *Am J Physiol Endocrinol Metab* 276:E596–E601.
- Schlue W-R, Dörner R (1992) The regulation of pH in the central nervous system. *Can J Physiol Pharmacol* 70:S278–S285.
- Schneider G-H, Baethmann A, Kempfski O (1992) Mechanisms of glial swelling induced by glutamate. *Can J Physiol Pharmacol* 70:S334–S343.
- Schousboe A (2003) Role of astrocytes in the maintenance and modulation of glutamatergic and GABAergic neurotransmission. *Neurochem Res* 28:347–352.
- Sernia C, Thomas WG (1994) A novel inhibitory role for glucocorticoids in the secretion of angiotensinogen by C6 glioma cells. *J Neurochem* 62:1296–1301.
- Silver IA, Erecińska M (1992) Ion homeostasis in rat brain in vivo: intra- and extracellular $[\text{Ca}^{2+}]$ and $[\text{H}^+]$ in the hippocampus during recovery from short-term transient ischemia. *J Cereb Blood Flow Metab* 12:759–772.
- Simard M, Couldwell WT, Zhang W, Song H, Liu S, Cotrina ML, Goldman S, Nedergaard M (1999) Glucocorticoids: potent modulators of astrocytic calcium signaling. *Glia* 28:1–12.
- Simard M, Arcuino G, Takano T, Liu QS, Nedergaard M (2003) Signaling at the gliovascular interface. *J Neurosci* 23:9254–9262.
- Simmons ML, Murphy S (1992) Induction of nitric oxide synthase in glial cells. *J Neurochem* 59:897–905.
- Simmons ML, Murphy S (1994) Roles for protein kinases in the induction of nitric oxide synthase in astrocytes. *Glia* 11:227–234.
- Smith SJ (1994) Neuromodulatory astrocytes. *Curr Biol* 4:807–810.
- Sonneward U, Westergaard N, Schousboe A (1997) Glutamate transport and metabolism in astrocytes. *Glia* 21:56–63.
- Sontheimer H (1992) Astrocytes, as well as neurons, express a diversity of ion channels. *Can J Physiol Pharmacol* 70:S223–238.
- Sontheimer H, Black JA, Waxman SG (1996) Voltage-gated Na^+ channels in glia: properties and possible functions. *Trends Neurosci* 19:325–331.
- Steinhauser C, Gallo V (1996) News on glutamate receptors in glial cells. *Trends Neurosci* 19:339–345.
- Stornetta RL, Hawelu-Johnson CL, Guyenet PG, Lynch KR (1988) Astrocytes synthesize angiotensinogen in brain. *Science* 242:1444–1446.
- Strange K, Emma F, Jackson PS (1996) Cellular and molecular physiology of volume-sensitive anion channels. *Am J Physiol* 270:C711–C730.
- Swanson RA (1992) Physiologic coupling of glial glycogen metabolism to neuronal activity in brain. *Can J Physiol Pharmacol* 70:S138–S144.
- Tallant EA, Higson JT (1999) Angiotensin II activates distinct signal transduction pathways in astrocytes isolated from neonatal rat brain. *Glia* 19:333–342.
- Tas PWL, Massa PT, Kress HG, Koschel K (1987) Characterization of an Na^+ , K^+ , Cl^- co-transport in primary cultures of astrocytes. *Biochim Biophys Acta* 903:411–416.
- Tashian RE (1989) The carbonic anhydrase: widening perspectives on their evolution, expression and function. *Bioessays* 10:186–192.
- Thiemann A, Grunder S, Push M, Jentsch TJ (1992) A chloride channel widely expressed in epithelial and non-epithelial cells. *Nature* 356:57–60.
- Troadec J-D, Thirion S, Petturiti D, Bohn MT (1999) ATP acting on P_{2y} receptors triggers calcium mobilization in primary cultures of rat neurohypophysial astrocytes (pituocytes). *Eur J Physiol* 437:745–753.
- Tuz K, Ordaz B, Vaca L, Quesada O, Pasantes-Morales H (2001) Isovolumetric regulation mechanisms in cultured cerebellar granule neurons. *J Neurochem* 79:143–151.
- Vajda Z, Pedersen M, Fuchtbauer E-M, Wertz K, Stødkilde-Jørgensen H, Sulyok E, Doczi T, Neely JD, Agre P, Frøhaier J, Nielsen S (2002) Delayed onset of brain edema and mislocalization of aquaporin-4 in dystrophin-null transgenic mice. *Proc Natl Acad Sci USA* 99:13131–13136.
- Venance L, Stella N, Glowinski J, Giaume C (1997) Mechanism involved in initiation and propagation of receptor-induced intercellular calcium signaling in cultured rat astrocytes. *J Neurosci* 17:1981–1992.
- Verkhatsky A, Steinhauser C (2000) Ion channels in glial cells. *Brain Res Rev* 32:380–412.
- Vezzani A, Wu HQ, Angelico P, Stasi MA, Samanin R (1988) Quinolinic acid-induced seizures, but not nerve cell death, are associated with extracellular Ca^{2+} decrease assessed in the hippocampus by brain dialysis. *Brain Res* 454:289–297.
- Vielkind U, Walencewicz A, Levine JM, Churchill Bohn M (1990) Type II glucocorticoid receptors are expressed in oligodendrocytes and astrocytes. *J Neurosci Res* 27:360–373.
- Vincent SR (1994) Nitric oxide a radical neurotransmitter in the central nervous system. *Prog Neurobiol* 42:129–160.
- Wadiche JI, Kavanaugh MP (1998) Macroscopic and microscopic properties of a cloned glutamate transporter/chloride channel. *J Neurosci* 18:7650–7661.
- Waller CL, Wyrick SD, Park HM, Kemp WE, Smith FT (1994) Effects of [(N-alkyl-1,3-dihydro-1-oxoisindolin-5-yl)oxy]alkanoic acids on chloride transport in primary astroglial cultures. *J Pharmacol Sci* 83:571–576.
- Waltz W (1995) Distribution and transport of chloride and bicarbonate ions across membranes. In: *Neuroglia* (Kettenmann H, Ransom B, eds), pp 221–229. New York: Oxford University Press.
- Weikert S, Freyer D, Weih M, Isaev N, Busch C, Schultze J, Megow D, Dirnagl U (1997) Rapid Ca^{2+} -dependent NO-production from central nervous system cells in culture measured by NO-nitrite/ozone chemoluminescence. *Brain Res* 748:1–11.
- Wetzel RK, Arystarkhova E, Sweadner KJ (1999) Cellular and subcellular specification of Na, K-ATPase α and β isoforms in the postnatal development of mouse retina. *J Neurosci* 19:9878–9889.
- Wilkins MR, Redondo J, Brown LA (1997) The natriuretic-peptide family. *Lancet* 349:1307–1310.

- Wolff JE, Lathera J, Goldstein GW (1992) Steroid inhibition of neural microvessel morphogenesis in vitro: receptor mediation and astroglial dependence. *J Neurochem* 58:1023–1032.
- Wright JW, Harding JW (1992) Regulatory role of brain angiotensins in the control of physiological and behavioral responses. *Brain Res Brain Res Rev* 17:227–262.
- Yang G, Thackery SG, Sigmund CD, Cassell MD (1999) The angiotensinogen gene is expressed in both astrocytes and neurons in murine central nervous system. *Brain Res* 817:123–131.
- Yang-Yen HF, Chambard JC, Sun YL, Smeal T, Schmidt TJ, Drouin J, Karin M (1990) Transcriptional interference between *c-jun* and the glucocorticoid receptor: mutual inhibition of DNA binding due to direct protein-protein interaction. *Cell* 62:1205–1215.
- Ye Z-C, Wyeth MS, Baltan-Tekkok S, Ransom BR (2003) Functional hemichannels in astrocytes: a novel mechanism of glutamate release. *J Neurosci* 23:3588–3596.
- Yu S, Ding W-G (1998) The 45 kDa form of glucose transporter 1 (GLUT1) is localized in oligodendrocyte and astrocyte but not in microglia in the rat brain. *Brain Res* 797:65–72.
- Zanotti S, Charles A (1997) Extracellular calcium sensing by glial cells: low extracellular calcium induces intracellular calcium release and intracellular signaling. *J Neurochem* 69:594–602.
- Zhang J-M, Wang H-K, Ye C-Q, Ge W, Chen Y, Jiang Z-L, Wu C-P, Poo M-M, Duan S (2003) ATP release by astrocytes mediates glutamatergic activity-dependent heterosynaptic suppression. *Neuron* 40:971–982.
- Zhu Y, Kimelberg HK (2001) Developmental expression of metabotropic P2Y(1) and P2Y(2) receptors in freshly isolated astrocytes from rat hippocampus. *J Neurochem* 77:530–541.
- Zhou M, Kimelberg HK (2000) Freshly isolated astrocytes from rat hippocampus show two distinct current patterns and different $[K^+]_o$ uptake capabilities. *J Neurophysiol* 84:2746–2757.
- Zonta M, Angulo MC, Gobbo S, Rosengarten B, Hossmann KA, Pozzan T, Carmignoto G (2003a) Neuron-to-astrocyte signaling is central to the dynamic control of brain microcirculation. *Nat Neurosci* 6:43–50.
- Zonta M, Sebelin A, Gobbo S, Fellin T, Pozzan T, Carmignoto G (2003b) Glutamate-mediated cytosolic calcium oscillations regulate a pulsatile prostaglandin release from cultured rat astrocytes. *J Physiol* 553:407–414.
- Zwain IH, Yen SSC, Cheng CY (1997) Astrocytes cultured in vitro produce estradiol-17 β and express aromatase cytochrome *P*-450 (*P*-450 AROM) mRNA. *Biochim Biophys Acta* 1334:338–348.

(Accepted 30 August 2004)
(Available online 11 November 2004)