65 Tamatani, M. *et al.* (1998) Roles of Bcl-2 and caspases in hypoxia-induced neuronal cell death: a possible neuroprotective mechanism of peptide growth factors. *Brain Res. Mol. Brain Res.* 58, 27–39
66 Bossenmeyer-Pourie, C. *et al.* (2000) Involvement of caspase 1 proteases in hypoxic brain injury. Effects of their inhibitors in developing neurons. *Neuroscience* 95, 1157–1165
67 Plata-Salaman, C. and Turrin, N. (1999) Cytokine interactions and cytokine balance in the brain: relevance to neurology and psychiatry. *Mol. Psychiatry* 4, 302–306
68 Muller, N. and Ackenheil, M. (1998) Psychoneuroimmunology and the cytokine action in the CNS: implications for psychiatric disorders. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* 22, 1–33
69 Pasinelli, P. *et al.* (1998) Caspase 1 is activated in neural cells and tissue with amyotrophic lateral sclerosis-associated mutations in copper-zinc superoxide dismutase. *Proc. Natl. Acad. Sci. U. S. A.* 95, 15763–15768
70 Li, M. *et al.* (2000) Functional role of caspase 1 and caspase-3 in an ALS transgenic mouse model. *Science* 288, 335–339
71 Huang, D. *et al.* (1998) Polymorphisms in IL-1β and IL-1 receptor antagonist genes are associated with myasthenia gravis. *J. Neuroimmunol.* 81, 76–81
72 Sciacca, F.L. *et al.* (1999) Relevance of interleukin 1 receptor antagonist intron 2 polymorphism in Italian MS patients. *Neurology* 52, 1896–1898
73 Martin, D. and Near, S.L. (1995) Protective effect of the interleukin-1 receptor antagonist (IL-1ra) on experimental allergic encephalomyelitis in rats. *J. Neuroimmunol.* 61, 241–245
74 De Simoni, M.G. *et al.* (2000) Inflammatory cytokines and related genes are induced in the rat hippocampus by limbic status epilepticus. *Eur. J. Neurosci.* 12, 2623–2633
75 Sheng, J.G. *et al.* (1994) Increased neuronal beta-amyloid precursor protein expression in human temporal lobe epilepsy: association with interleukin-1α immunoreactivity. *J. Neurochem.* 63, 1872–1879
76 Kanemoto, K. *et al.* (2000) Interleukin (IL)1β, IL-1α, and IL-1 receptor antagonist gene polymorphisms in patients with temporal lobe epilepsy. *Ann. Neurol.* 47, 571–574
77 Mehler, M.F. and Kessler, J.A. (1997) Hematolymphopoietic and inflammatory cytokines in neural development. *Trends Neurosci.* 20, 357–365
78 Dziegielewska, K.M. *et al.* (2000) Acute-phase cytokines IL-1β and TNF-α in brain development. *Cell Tissue Res.* 299, 335–345
79 Ilyin, S.E. *et al.* (2000) Autoregulation of the interleukin-1 system and cytokine-cytokine interactions in primary human astrocytoma cells. *Brain Res. Bull.* 51, 29–34
80 Gayle, D. *et al.* (1997) Central nervous system IL-1β system and neuropeptide Y mRNAs during IL-1β-induced anorexia in rats. *Brain Res. Bull.* 44, 311–317
81 Plata-Salaman, C.R. *et al.* (1998) Gram-negative and gram-positive bacterial products induce differential cytokine profiles in the brain: analysis using an integrative molecular-behavioral *in vivo* model. *Int. J. Mol. Med.* 1, 387–397
82 Yao, J. and Johnson, R.W. (1997) Induction of interleukin-1 β-converting enzyme (ICE) in murine microglia by lipopolysaccharide. *Brain Res. Mol. Brain Res.* 51, 170–178
83 Tingsborg, S. *et al.* (1997) Regulation of ICE activity and ICE isoforms by LPS. *Mol. Psychiatry* 2, 122–124
84 Laye, S. *et al.* (1996) Effects of lipopolysaccharide and glucocorticoids on expression of interleukin-1β converting enzyme in the pituitary and brain of mice. *J. Neuroimmunol.* 68, 61–66
85 O'Neill, L.A. and Dinarello, C.A. (2000) The IL-1 receptor/toll-like receptor superfamily: crucial receptors for inflammation and host defense. *Immunol. Today* 21, 206–209
86 Hagberg, H. *et al.* (1996) Enhanced expression of interleukin (IL)-1 and IL-6 messenger RNA and bioactive protein after hypoxia-ischemia in neonatal rats. *Pediatr. Res.* 40, 603–609
87 Szaflarski, J. *et al.* (1995) Cerebral hypoxia-ischemia stimulates cytokine gene expression in perinatal rats. *Stroke* 26, 1093–1100
88 Bossenmeyer-Pourie, C. *et al.* (2000) Involvement of caspase 1 proteases in hypoxic brain injury. Effects of their inhibitors in developing neurons. *Neuroscience* 95, 1157–1165
89 Liu, X.H. *et al.* (1999) Mice deficient in interleukin-1 converting enzyme are resistant to neonatal hypoxic-ischemic brain damage. *J. Cereb. Blood Flow Metab.* 19, 1099–1108
90 Legos, J.J. *et al.* (2000) Quantitative changes in interleukin proteins following focal stroke in the rat. *Neurosci. Lett.* 282, 189–192
91 Sanz, J.M. and Virgilio, F.D. (2000) Kinetics and mechanism of ATP-dependent IL-1β release from microglial cells. *J. Immunol.* 164, 4893–4898
92 Laliberte, R.E. *et al.* (1999) ATP treatment of human monocytes promotes caspase 1 maturation and externalization. *J. Biol. Chem.* 274, 36944–36951
93 Goujon, E. *et al.* (1997) Regulation of cytokine gene expression in the central nervous system by glucocorticoids: mechanisms and functional consequences. *Psychoneuroendocrinology* 22 (Suppl. 1), S75–S80
94 Flower, R.J. and Rothwell, N.J. (1994) Lipocortin-1: cellular mechanisms and clinical relevance. *Trends Pharmacol. Sci.* 15, 71–76
95 Smith, E.M. *et al.* (1999) IL-10 as a mediator in the HPA axis and brain. *J. Neuroimmunol.* 100, 140–148
96 Satoh, J. *et al.* (1998) Constitutive and cytokine-inducible expression of prion protein gene in human neural cell lines. *J. Neuropathol. Exp. Neurol.* 57, 131–139
97 Pousset, F. *et al.* (1999) Interleukin-4 and interleukin-10 regulate IL-1β induced mouse primary astrocyte activation: a comparative study. *Glia* 26, 12–21

ATP: an extracellular signaling molecule between neurons and glia

R. Douglas Fields and Beth Stevens

Recent studies on Schwann cells at the neuromuscular junction and non-synaptic regions of premyelinated axons indicate that extracellular ATP can act as an activity-dependent signaling molecule in communication between neurons and glia. Several mechanisms have been observed for the regulated release of ATP from synaptic and non-synaptic regions, and a diverse family of receptors for extracellular ATP has been characterized. The findings suggest functional consequences of neuron–glial communication beyond homeostasis of the extracellular environment surrounding neurons, including regulating synaptic strength, gene expression, mitotic rate, and differentiation of glia according to impulse activity in neural circuits.

Trends Neurosci. (2000) 23, 625–633

R. Douglas Fields is head of the Neurocytology and Physiology Unit, Laboratory of Developmental Neurobiology at the National Institutes of Health, NICHD, Bldg 49, Room 5A38, 49 Convent Drive, Bethesda, MD 20892, USA. Beth Stevens is at the National Institutes of Health and at the University of Maryland, College Park, Neuroscience and Cognitive Science Program, Dept of Biology, College Park, MD 20892, USA.

THE REALIZATION that neural impulses and synaptic activity can influence glial function has emerged from experiments on all the major types of glia in the PNS and CNS of vertebrates and invertebrates. It has also become clear that glia can respond to these signals in ways that regulate the excitability of neurons. With the discovery that glia, similar to neurons, also have ion channels and neurotransmitter receptors, attention

PII: S0166-2236(00)01674-X

first focused on extracellular ions and neurotransmitters as possible signaling molecules in neuron–glial communication. However, it is known that all types of glia (i.e. microglia, oligodendrocytes, astrocytes and Schwann cells) have membrane receptors for extracellular ATP (purinergic receptors) (Table 1). These receptors are linked by G-coupled proteins to intracellular Ca^{2+} release channels or to transmembrane Ca^{2+} channels. Additionally, it is known that many neurons can release ATP in an activity-dependent manner. Research on Schwann cells, the PNS glial cell, at both the synapse[1] and in non-synaptic regions[2] shows that ATP can act as a potent activity-dependent signaling molecule between neurons and glia. Thus, multiple mechanisms for ATP release and the widespread distribution of purinergic receptors throughout the nervous system indicate that ATP might mediate neuron–glial signaling more generally and in association with a variety of functions. Diffusion of neurotransmitter beyond the synaptic cleft can activate neurotransmitter receptors on perisynaptic astrocytes and terminal Schwann cells (reviewed in Ref. 3); and changes in extracellular ion concentration accompanying action potential firing in extrasynaptic regions, such as the nodes of Ranvier, can cause Ca^{2+} transients in paranodal Schwann cells[4]. However, glial responses to these kinds of molecules are consistent with their well-established function in regulating the concentration of ions and neurotransmitters in the extracellular environment. By contrast, the findings indicating that ATP can act as a neuron–glial signaling molecule expand the functional significance of activity-dependent neuron–glial communication beyond processes associated with homeostasis of the extracellular environment surrounding neurons. For example, profound effects on Schwann cell gene expression, mitotic rate and differentiation have been identified in response to activity-dependent release of ATP from non-synaptic regions of premyelinated dorsal root ganglion (DRG) neurons. Such effects could be important in coordinating the development of neurons and glia according to functional requirements[2]. Several questions are raised by these results including, how general is the mechanism of intercellular communication by ATP? What are the functional consequences of this communication? How is ATP released in an activity-dependent manner?

Neuron–glial signaling at the synapse

Glial receptors for the vesicular release of neurotransmitters

Astrocytes in the CNS and Schwann cells in the PNS surround synaptic junctions and help maintain the extracellular environment by providing physical integrity, and regulating the extracellular ion and neurotransmitter concentration. Ca^{2+} imaging, molecular, and electrophysiological methods show that, under appropriate conditions, oligodendrocyte precursor cells (OPC), astrocytes and Schwann cells can detect the vesicular release of neurotransmitters. The stimulated glia can subsequently regulate synaptic strength by releasing neurotransmitters, such as glutamate, into the synaptic cleft[3].

A recent study reveals a surprisingly sophisticated level of neuron–glial communication via the vesicular release of neurotransmitters[5]. Using whole-cell patch-clamp recordings from OPCs in the CA1 region of hippocampus, rapid transmembrane currents were detected in response to stimulation of afferent excitatory axons (i.e. the Schaffer collateral axons of CA3 pyramidal neurons). AMPA/kainate receptor antagonists block these currents in OPC cells, however it is improbable that spillover of glutamate from nearby neuronal synapses could produce glial responses with such rapid kinetics. In addition, ultrastructural analysis was used to show clusters of synaptic vesicles, resembling presynaptic transmitter release sites, directly apposed to OPC processes, and 'postsynaptic' membrane specializations in the adjacent glial membrane.

The functional significance of this neuron–glial communication is unknown; however, OPCs can extend processes into the region of axo-spinous synapses, suggesting that these glial cells could modulate neuronal synaptic transmission, similar to perisynaptic astrocytes. Glutamate is released from astrocytes by a mechanism similar to vesicular release in neurons: release is dependent on extracellular Ca^{2+}, synaptic vesicle docking and release proteins[6]. Glutamate that is secreted from perisynaptic astrocytes can augment the release of neurotransmitter into the synaptic cleft by the presynaptic neuron[7,8]. Conversely, neuron–glial communication might provide a mechanism for axonal activity to regulate oligodendrocyte functions, such as myelination, which has been shown to be regulated by impulse activity in the CNS (Ref. 9) and PNS (Ref. 10).

In the PNS, terminal Schwann cells, which tightly surround the neuromuscular junction, actively participate in the maintenance and repair of neuromuscular synapses[11]. Tetanic stimulation of the presynaptic motor nerve axon elicits rapid Ca^{2+} transients in terminal Schwann cells of frogs[12] and mice[13], and downregulates expression of glial fibrillary acidic protein (GFAP) (Ref. 14). This activity-dependent increase in Ca^{2+} is reduced if neurotransmitter receptors are blocked, and can be mimicked by focal application of the transmitter ACh (Ref. 12). Elevation of Ca^{2+} levels is caused by transmembrane Ca^{2+} fluxes through ligand- and voltage-gated channels, and release from intracellular stores following activation of muscarinic ACh receptors[1]. In addition, perisynaptic Schwann cells can, in turn, modulate neurotransmitter release and synaptic efficacy at the frog neuromuscular junction. In a recent study, Robitaille *et al.*[15] showed that microinjection of GTPγS into perisynaptic Schwann cells reduced the amount of neurotransmitter released from the neuromuscular junction. This finding indicates that activation of a G-coupled protein in Schwann cells stimulates the release of a retrograde messenger that can act upon the presynaptic terminal. Although the molecular mechanisms are unclear, Schwann cells are known to release neuroactive substances that could modulate synaptic transmission[16]. Because purinergic receptors are pertussis toxin sensitive and this toxin did not block the effect of GTPγS Robitaille *et al.*[15] concluded that activation of muscarinic receptors, and not purinergic receptors, on Schwann cells mediates the response.

ATP mediated neuron–glial signaling

Many properties of ATP make it an ideal molecule for cell–cell signaling: it is a small, rapidly diffusing molecule, highly unstable and not abundant in the extracellular environment. Extracellular ATP has been implicated in cell–cell communication outside the nervous system in a wide variety of cells in response to many different stimuli associated with a diverse array of biological effects (reviewed in Ref. 17). ATP or the

breakdown products of ATP can influence epithelial and endocrine cell secretion, leukocyte adhesion, immune, inflammatory and thrombotic reactions, cardiovascular performance, skeletal and smooth muscle contraction, and neurotransmission[18]. For example, in the cochlea, extracellular ATP has five verified actions that affect sound transduction by the sensory hair cells, ranging from acting as a neurotransmitter to regulating mechanical stiffness of the stereocilia (reviewed in Ref. 19). Mechanical stimulation, hypoxia, acidosis, osmotic shock, receptor stimulation and membrane depolarization can all induce ATP release from various cells[18].

Release mechanisms, similar to those originally described in cells outside the nervous system, appear to apply to ATP release by neurons and glia. Mast cells, for example, have Fc membrane receptors that trigger secretion of inflammatory mediators following stimulation. This is accompanied by an increase in intracellular Ca^{2+}, not only in the stimulated cell, but also spreading radially into neighboring cells. Similar to astrocytes, this intercellular Ca^{2+} wave can be initiated by mechanical stimulation of a single cell, however, mast cells are not coupled by gap junctions. Experiments by Osipchuk and Cahalan[20] have shown that ATP co-released with mast-cell secretory granules triggers the Ca^{2+} response.

Recently, ATP release by mechanically stimulated astrocytes has been identified as a key signaling molecule in astrocytic Ca^{2+} waves[21], resolving a controversy that has persisted since the waves were first observed in response to glutamate stimulation[22]. Ca^{2+} diffusion through gap junctions and extracellular signaling molecules had been proposed as key signaling molecules for astrocytic Ca^{2+} waves and it appears that both could contribute. Ca^{2+} wave propagation in dorsal spinal cord astrocytes is mediated by P2Y receptors[23], and in the mammalian retina ATP generates intracellular Ca^{2+} waves that propagate through networks of glial cells[24]. The firing rate of retinal neurons is affected by the passage of a glial Ca^{2+} wave[25], suggesting that ATP-mediated signaling in perisynaptic glia could regulate the excitability of neurons or the synaptic transmission by releasing neurotransmitters. ATP has also been shown to stimulate the release of excitatory amino acids from cultured Schwann cells[26] and astrocytes[3,21]. The secretion of glutamate from astrocytes is vesicular, and dependent on extracellular Ca^{2+}, and the synaptic vesicle protein 25 kDa synaptosomal-associated protein (SNAP-25) (Ref. 6). However, the release of ATP from astrocytes might not be vesicular, because Ca^{2+} waves propagate among astrocytes in the presence of botulinum toxin[6].

Vesicular release of ATP

Vesicular release of ATP is involved in excitatory transmission in CNS[27] and PNS[28] neurons. ATP is co-released with ACh and noradrenaline in the PNS (Ref. 29), and with GABA from dorsal horn neurons[30]. Neuronal purinergic receptors (Table 1) of the P2X ionotropic subtype are widely distributed in the nervous system, in addition to adenosine receptors that bind this breakdown product of ATP (Ref. 31). In cultured rat sympathetic neurons for example, ATP (100 μM) can cause depolarization, action potential firing, influx of Ca^{2+} through P2X receptors and voltage-gated Ca^{2+} channels, and stimulates release of noradrenaline[32].

What could be the purpose of releasing ATP, together with neurotransmitter, from synaptic vesicles? This question has been most extensively studied in motor nerve endings, where ATP is released together with ACh. Using patch-clamp recording on membranes containing ATP-gated ion channels receptors (P2X) as biosensors, Silinsky *et al.*[33] detected the quantal release of ATP within milliseconds of a nerve impulse. After release, ATP is rapidly hydrolyzed to adenosine by ectonucleotidases. Similar to other neurotransmitters, removal of ATP helps terminate its response, but, in addition, adenosine then acts on presynaptic adenosine receptors to inhibit neurotransmitter release and depress synaptic transmission at the neuromuscular junction[34].

Similar effects are seen in the CNS. In cultured chick retinal neurons (amacrine cells) ATP release is stimulated by depolarization, which is dependent on extracellular Ca^{2+}, activation of L and P/Q type voltage-sensitive Ca^{2+} channels, and the synaptic protein SNAP-25 (Ref. 35). This results in extracellular accumulation of adenosine, an effect that is partly antagonized by inhibiting ectonucleotidase activity. ATP-mediated synaptic transmission also occurs in other cholinergic regions of the CNS, including the hippocampus (reviewed in Ref. 36).

Purinergic receptors on glial cells provide a potential mechanism for detecting the synaptic activity of neurons using ATP as a neurotransmitter. Studies on the frog neuromuscular junction provide support for ATP-dependent neuron–glial signaling[12], and subsequent regulation of synaptic transmission via release of an unknown factor from the terminal Schwann cell[1]. Perisynaptic Schwann cells express several types of purinergic receptors, including P2X and P2Y, in addition to adenosine P1 receptors (Table 1). Blockade of ATP, but not of adenosine, significantly reduces the size and increases the delay of the activity-dependent Ca^{2+} increase in terminal Schwann cells, suggesting that purinergic receptors on perisynaptic Schwann cells are activated by ATP that is released by synaptic transmission[1]. Although *in situ* characterization of the P2 receptors on these cells is not complete, the presence of multiple purinergic receptors suggests that ATP could activate multiple signaling pathways and have diverse functional effects on synaptic strength, maintenance and remodeling.

Extrasynaptic neuron–glial signaling

Activity-dependent interactions between neurons and glia in extrasynaptic regions would encompass a large range of functions, including myelination and various glial functions unrelated to synaptic transmission (e.g. proliferation and differentiation). Time-lapse confocal microscopy has recently shown that Schwann cells in culture can respond to electrical stimulation of premyelinated DRG axons[2] (Fig. 1). This is particularly interesting because these neurons lack synapses and nodes of Ranvier in culture. Trains of stimulation of about 15–90 s were required to induce Ca^{2+} responses in Schwann cells, proportional to stimulus frequency (1–10 Hz). By contrast to astrocytes, direct application of glutamate failed to induce a Ca^{2+} response in Schwann cells[2].

Based on evidence showing the presence of purinergic receptors on myelinating and non-myelinating Schwann cells *in vivo* (Table 1), we analyzed the concentration of extracellular ATP in DRG neuron cultures. They showed that electrical stimulation of pure DRG neuron cultures significantly increased the concentration of ATP in the

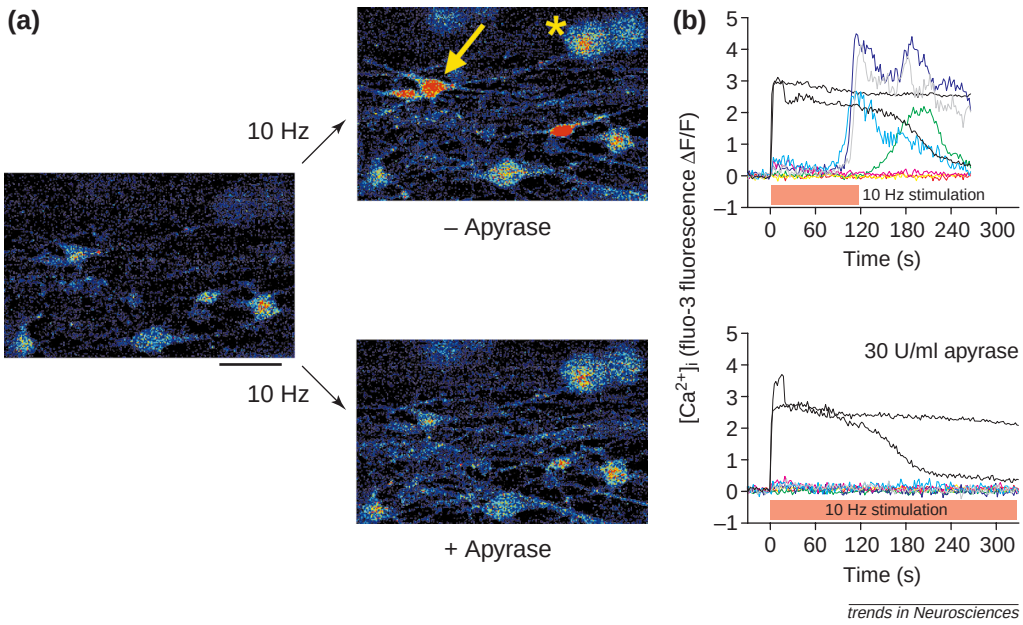

Fig. 1. ***Axonal impulse activity stimulates Schwann cells on premyelinated dorsal root ganglion axons by releasing ATP.*** **(a)** *Scanning-laser confocal microscopy was used to monitor changes in intracellular Ca^{2+} with the fluorescent indicator fluo-3/AM in co-cultured Schwann cells and dorsal root ganglion (DRG) neurons. Electrical stimulation of DRG axons at 10 Hz caused an immediate increase in intracellular Ca^{2+} in the axon and cell body (asterisk), followed several seconds later by a large increase in Ca^{2+} in Schwann cells (arrow). (The blue 'cool' colors represent low intracellular Ca^{2+} levels and the red 'warm' colors indicate high intracellular Ca^{2+} concentrations.)* **(b)** *The upper plot shows changes in intracellular Ca^{2+} concentration $[Ca^{2+}]_i$ over time. The lower plot shows the changes in $[Ca^{2+}]_i$ when the experiment was repeated in the presence of apyrase (an extracellular enzyme that rapidly degrades ATP) following a 30 min rest. The stimulus duration in both plots is shown by the red bar (black traces, neurons; color traces, Schwann cells). Apyrase had no effect on electrically-induced responses in neurons, but blocked the response in Schwann cells, indicating that extracellular ATP is crucial in this activity-dependent neuron–glial signaling. Scale bar, 50 μm.* (b) Reproduced, with permission, from Ref. 2.

culture medium, in spite of the absence of synapses. By contrast, ATP secretion was not induced by electrical or KCl depolarization of Schwann cells in the absence of neurons. In co-cultures of Schwann cells and DRG neurons, the rise in intracellular Ca^{2+} in Schwann cells was blocked when axons were stimulated in the presence of apyrase, an enzyme that rapidly degrades extracellular ATP (Fig. 1). This indicates that although action potentials might induce the release of many different substances, the Ca^{2+} response in Schwann cells in these experiments is induced by the activity-dependent secretion of ATP from non-synaptic regions of DRG neurons. Electrical stimulation of DRG neurons at 10 Hz subsequently activates Ca^{2+}-calmodulin kinase type II (CaMKII), which phosphorylates the transcription factor cAMP-response-element-binding protein (CREB) and stimulates increased mRNA and protein expression of the immediate early genes *fos* and *krox* 24; genes involved in adaptive responses and differentiation of Schwann cells.

In the adult, activity-induced Ca^{2+} transients in Schwann cells have also been observed along non-synaptic areas of myelinated axons in frog sciatic nerve in response to a 5 min 20 Hz stimulation[4]. Presumably this effect is mediated by accumulation of extracellular K^+ at the node. Reist and Smith[13] measured Ca^{2+} responses in terminal Schwann cells following a 10–20 s stimulation at 50 Hz, and observed no changes in intracellular Ca^{2+} in proximal myelinated Schwann cells, possibly because longer stimulation times are required for accumulation of K^+ in the extracellular space. In the rat vagus and human sural unmyelinated nerves, Grafe and colleagues[37] did not observe Ca^{2+} responses in Schwann cells following a 1–50 Hz stimulation for 5 s. Together, these results show that Ca^{2+} responses in Schwann cells are dependent on the duration of axonal stimulation, suggesting that different signaling molecules might be activated by different stimulus paradigms.

In the CNS, non-synaptic activity-dependent signaling has also been shown in the developing rat optic nerve before the onset of myelination. Repetitive axonal stimulation (10–20 Hz) elicits Ca^{2+} transients in approximately 20% of optic nerve glia 15–60 s after stimulation in a frequency-dependent manner[38]. This response is blocked with TTX and occurs in the absence of extracellular Ca^{2+} suggesting the non-vesicular release of a neuroactive substance. Non-vesicular release of adenosine[39] and glutamate[40] from central and peripheral axons has been shown in response to action potentials.

Could ATP contribute to activity-dependent communication in optic nerve glia and Schwann cells of adult peripheral nerve? Astrocytes and oligodendrocytes, and myelinated, non-myelinated, and paranodal Schwann cells all express purinergic receptors (Table 1). The lack of response to electrical stimulation in unmyelinated adult Schwann cells (Ref. 37) could be as a result of insufficient stimulus duration to evoke ATP release, however differences in ATP release mechanisms in developing and adult nerve might also exist.

Non-vesicular ATP release in extrasynaptic neuron–glial signaling

The mechanisms for the activity-dependent release of ATP from non-synaptic regions of premyelinated DRG neurons are unknown. Stimulation of ATP secretion is blocked in the presence of TTX, eliminating electrolytic axon injury as a mechanism[2]. Secretion via ATP transporters or channels, or the vesicular release in non-synaptic regions are all viable mechanisms that remain to be tested. In some non-neuronal cells ATP can be released via plasma-membrane-transport proteins, and possibly ATP-permeable channels. In addition, the cystic fibrosis transmembrane conductance regulator (CFTR) (Ref. 41), P-glycoprotein (Ref. 42), and other members of the ATP-binding cassette (ABC) family of transporters are involved in ATP release from cells. In recent experiments, ATP efflux through the CFTR was not detected[43,44], but ATP release involving these receptors has been reported to be under control of both cAMP activation and a change in the Cl^- gradient[45].

Recent experiments on astrocytes suggest that ATP might be released by gap-junction hemichannels[46]. Connexin hemichannels can open in response to membrane voltage or to a reduction in extracellular Ca^{2+} concentration[47,48]; this might provide a means for ATP efflux through the large diameter pores of these channels. In support of this hypothesis, increased ATP release from an astrocyte cell line is associated with transfection of connexin[49]. If this were the case, it

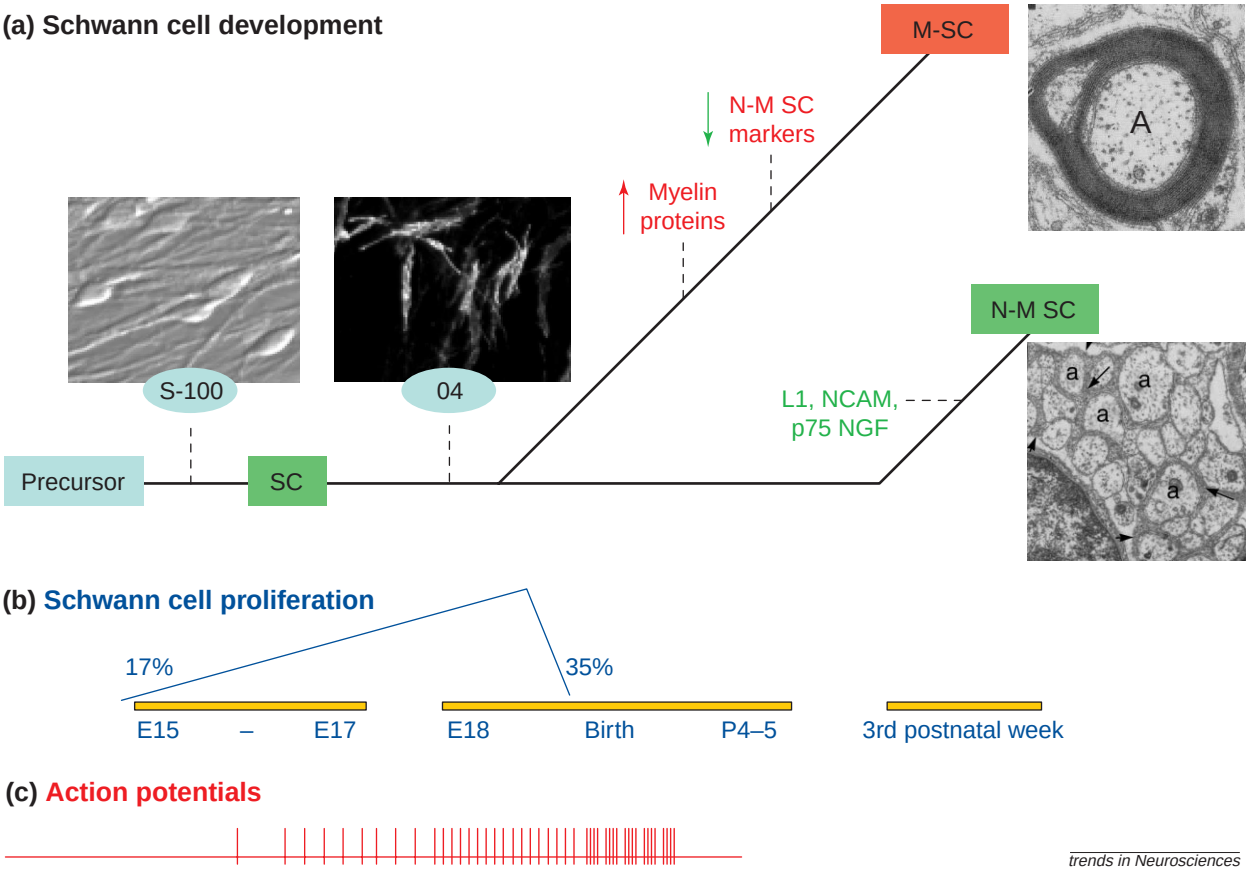

Fig. 2. ***Correlation between Schwann cell development and changes in neural impulse activity in dorsal root ganglion neurons of mouse during the perinatal period.*** **(a)** *Schwann cells precursors migrate out with the neural crest and begin to express the S-100 antigen. As they develop into immature Schwann cells they begin to express the 04 antigen, and then differentiate into either myelinating (M SC) or non-myelinating phenotypes (NM SC)*[52]. **(b)** *The rate of Schwann cell proliferation increases in late fetal development and begins to decrease near the time of birth*[51]. **(c)** *Action potentials from dorsal root gangloin (DRG) neurons show the onset of active spontaneous and sensory-evoked activity in DRG neurons coincides with the decrease in Schwann cells proliferation and differentiation*[52]. *ATP that is released by DRG neurons in culture inhibits Schwann cells proliferation and arrests development at a stage before development of the 04 antigen*[2]. *These correlations have yet to be tested* in vivo, *but suggest that impulse activity could stop proliferation and prevent terminal differentiation of Schwann cells until exposure to appropriate axon-specific differentiation signals.* Adapted, with permission, from Ref. 51 and Ref. 68.

would open up new possibilities for research, because gap junctions are widely expressed in the nervous system between many types of cells, including pairs of neurons, pairs of glia, and between neurons and glia. The conductance and developmental expression of gap junctions are highly regulated. Furthermore, it has been proposed that gap junctions could promote the activity-dependent organization of neurons into functional assemblies, even before synaptogenesis[50]. It is intriguing to speculate that ATP acting on neurons and glia might have a role in regulating nervous system development via activity-dependent extrasynaptic communication.

The activity-dependent axon–Schwann cell signaling studied in premyelinated DRG neurons suggests functional consequences that might be relevant to nervous system development (Fig. 2). In the perinatal period, Schwann cells undergo a reduction in proliferation and differentiate into either myelinating or non-myelinating phenotypes[51]. This developmental stage coincides with the onset of active spontaneous and stimulus-evoked impulse activity in DRG axons[52] (Fig. 2c). Experiments in co-culture show that the mitotic rate of Schwann cells is inhibited significantly on axons firing action potentials, and this can be mimicked by direct application of ATP or prevented by stimulation in the presence of apyrase (an extracellular enzyme that rapidly degrades ATP; Ref. 2). Interestingly ATP has previously been shown to affect the mitotic rate of other cells[53], including astrocytes[54]. In these cases, however, ATP stimulates, as oppose to inhibits, mitosis. The cellular mechanism for these different mitotic responses are, as yet, unknown, but differences in receptors or intracellular signaling pathways in Schwann cells could account for this.

ATP-mediated neuron–Schwann cell signaling can also strongly regulate Schwann cell lineage progression and differentiation[2]. Following proliferation Schwann cells begin differentiating into myelinating and non-myelinating phenotypes[51] (Fig. 2). Electrical stimulation of premyelinated DRG axons or ATP application, arrests the development of Schwann cells at a stage before expression of the developmental marker 04. Even after prolonged application of ATP (two weeks) in a medium promoting differentiation, Schwann cells remained arrested in this undifferentiated state, and failed to generate myelin-basic protein or form compact myelin compared with control cultures[2]. Instead of promoting differentiation towards a particular pathway, in this case impulse activity might serve as a signal to Schwann cells along the developing axon, indicating the appropriate time to exit the cell-cycle and become responsive to factors controlling differentiation. This could increase the pool of Schwann cells that are in a pre-differentiated

Box 1. Activity-dependent neuron–glial communication in synaptic and non-synaptic regions

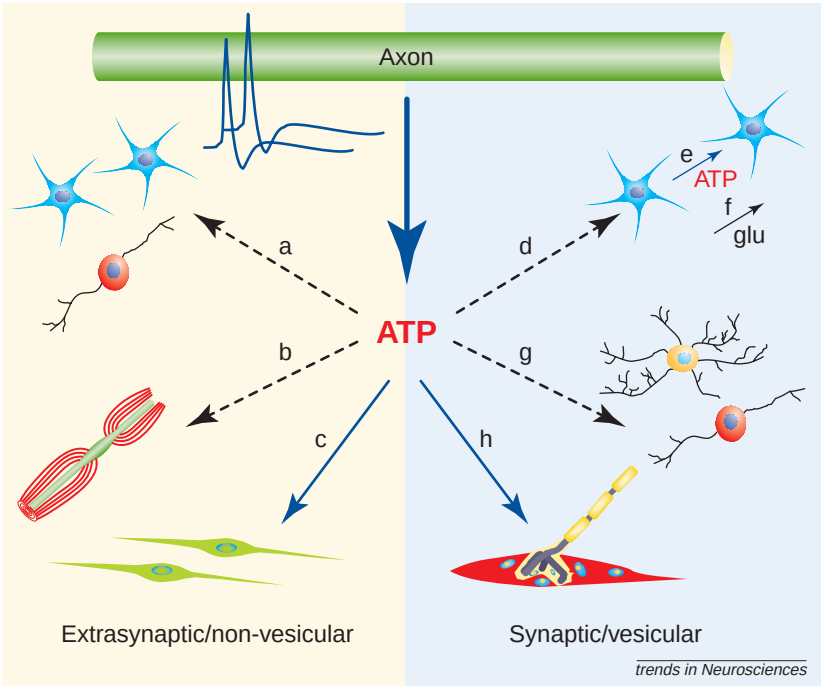

Fig. I. ***Activity-dependent neuron–glial communication.*** *Lettered arrows (a–h) represent actual or putative routes of ATP-mediated communication from the axon to glial cells in extrasynaptic (left) and synaptic (right) regions. Each of the letters also corresponds to the reference for that neuron–glial communication. Solid arrows indicate activity-dependent neuron–glial signaling mediated by ATP, dashed arrows indicate that involvement of ATP has not yet been shown.*

Impulse activity can be communicated to glia in synaptic and non-synaptic regions of the PNS and CNS via neurotransmitters, ions and ATP (Fig. I). Vesicular and non-vesicular release of ATP has been shown to mediate activity-dependent communication between neurons and Schwann cells in synaptic[f] and non-synaptic regions[c]. These findings, together with evidence showing that ATP mediates astrocyte–astrocyte signaling[e], suggests several possibilities for future research on a more general role of ATP in neuron–glial signaling.

References

a Kriegler, S. and Chiu, S.Y. (1993) Calcium signaling of glial cells along mammalian axons. *J. Neurosci.* 13, 4229–4245

b LevRam, V. and Ellisman, M.H. (1995) Axonal activation-induced calcium transients in myelinating Schwann cells, sources and mechanisms *J. Neurosci.* 15, 2628–2637

c Stevens, B. and Fields, R.D. (2000) Response of Schwann cells to action potentials in development. *Science* 287, 2267–2271

d Dani, J.W. *et al.* (1992) Neuronal activity triggers calcium waves in hippocampal astrocyte networks. *Neuron* 8, 429–440

e Guthrie, P.B. *et al.* (1999) ATP release from astrocytes mediates glial calcium waves. *J. Neurosci.* 19, 520–528

f Cornell-Bell, A.H. *et al.* (1990) Glutamate induces calcium waves in cultured astrocytes: long-range glial signaling. *Science* 247, 470–473

g Bergles, D.E. *et al.* (2000) Glutamatergic synapses on oligodendrocyte precursor cells in the hippocampus. *Nature* 405, 187–191

h Robitallie, R. (1995) Purinergic receptors and their activation by endogenous purines at perisynaptic glial cells of the frog neuromuscular junction. *J. Neurosci.* 15, 7121–7131

state and available to respond to appropriate myelination signals when they develop. Many myelination signals are axon-specific, and similar to the caliber of the axon, are related to maturation of the individual axon, which might not develop until the postnatal period.

In an interesting parallel in the CNS, glutamate receptor activation of OPCs inhibits their proliferation and maturation in culture[55], suggesting the possibility of a similar activity-dependent inhibitory effect on OPC development on glutamatergic neurons. Further research will be required to determine if ATP has a similar effect on OPCs.

Impulse activity can also have a positive effect on OPC proliferation, as shown by intraocular injection of TTX (Ref. 56). These effects of impulse activity blockade operate via mechanisms involving an indirect action of neural impulse activity on astrocytes (through an unknown signaling molecule) that in turn releases a trophic substance, such as platelet-derived growth factor (PDGF), that acts upon OPCs. Collectively, these studies show that there are multiple mechanisms and effects of activity-dependent neuron–glial signaling in extrasynaptic regions that are operational at least during limited periods of nervous system development.

It is curious that Schwann cells in adult nerve have P2 receptors that respond to exogenous ATP (Ref. 57), but show no response to action potential firing in other studies[37,58], in spite of observable changes in intracellular Ca^{2+} in paranodal[4] or terminal Schwann cells (Ref. 13) as a result of K^+ or neurotransmitter release. Indeed, intracellular Ca^{2+} responses in the terminal Schwann cells of skate electrocytes have been shown to involve P2 receptor stimulation in response to ATP released by K^+ depolarization[59]. Although technical limitations cannot be ruled out, the ATP release mechanism might be different during fetal development when these responses can be elicited in Schwann cells on premyelinated axons[2]. DRG neurons are electrotonically coupled early in development, but they become uncoupled as development progresses[60]. If these channels are only expressed at certain times in development and the hemichannels are able to release ATP into the extracellular medium, this could restrict ATP-mediated neuron–glial signaling to appropriate phases of development.

ATP release in neurotrauma

Cellular damage can release large amounts of ATP into the extracellular environment because the internal concentration of ATP can be between 3–5 mM (Refs 29,34). Such ATP release might be important in triggering cellular responses to trauma and ischemia, by initiating and maintaining reactive astrogliosis, which involves striking changes in astrocyte proliferation and morphology[54,61]. Nucleosides and nucleotides that are released from dying cells also stimulate proliferation of microglia (reviewed in Ref. 62). ATP release from astrocytes also has morphological effects on co-cultured cortical neurons, resulting in extension of longer neurites[49].

Not all the responses to ATP released during brain injury are neuroprotective; in some cases ATP contributes to the pathophysiology initiated after trauma. Treatment of cultured astrocytes with cytokines, such as interleukin-1β, enhances the ATP-evoked release of arachidonic acid via $P2Y_2$ receptors and cytosolic phospholipase A2. This

Box 2. Purinergic receptors

A large family of purinergic receptors mediate the various cellular effects of extracellular ATP. The receptors have been detected on many types of cells, including endocrine, hepatic cells, macrophages, platelets, fibroblasts and epithelia. In the nervous system, purinergic receptors are found on neurons, astrocytes, microglia, oligodendrocytes and Schwann cells. These receptors are classified broadly into two groups, based on their sensitivity to ATP (P2) or adenosine (P1) (Table 1). Adenosine receptors are divided into two main groups: A1, which inhibits adenylate cyclase and A2, which facilitates it. More recent molecular, biochemical and pharmacological evidence would further subdivide the adenosine receptors into four subtypes[dd]; however, this has only recently been applied to the glial literature in association with studies in astrocytes. P2 receptors are divided into metabotropic receptors (P2Y), which are linked to G-coupled proteins and ionotropic receptors (P2X). Several P2 receptor subtypes have been characterized at the molecular level and based on their sensitivity to agonists and antagonists.

TABLE 1. Purinergic receptors in dorsal root ganglion neurons and glia[a]

Cell type[c]	Purinergic receptor					
	P1(Adenosine)[b]		P2X (ATP, ionotropic)		P2Y (ATP-metabotropic)	
	Subtype	Ref.	Subtype	Ref.	Subtype	Ref.
Astrocyte	A_1 A_2	**a**,**b*** **a**,**c**	P2X $P2X_1$ $P2X_7$ (P2Z)	d e **f**	P2Y $P2Y_1$ $P2Y_2$ (P2U) $P2Y_4$	**g** **h**,**i**,**k** **g**,**i–k** **k**
Oligodendrocyte/ OPC					P2	**l**
Schwann cell (non-myelinating)					P2Y $P2Y_1$ $P2Y_2$ (P2U)	**m*** n **o**
Schwann cell (myelinating/ paranodal)			$P2X_7$	p	P2Y $P2Y_2$	**m** n
Perisynaptic Schwann cell	A_1	q	P2X	q	P2Y	q,r
Microglia	A_1 A_2	**s** **s**,**t**	$P2X_7$ (P2Z)	u,**v**	P2Y	u
DRG neurons	A_1	**w**,x	P2X $P2X_2$ $P2X_3$	**y**,**z** aa aa,bb	P2Y	**y**,cc

[a]Bold letters indicate studies done in culture. Studies carried out both *in situ* and in cell culture are indicated by an asterisk.

[b]More recent molecular, biochemical and pharmacological evidence would further subdivide the adenosine receptors into four subtypes (dd); however, this has only recently been applied in the glial literature in association with studies in astrocytes.

[c]Abbreviations: DRG, dorsal root ganglion; OPC, oligodendrocyte precursor cells.

References

a Ogata, T. *et al.* (1996) Potentiated cAMP rise in metabotropically stimulated rat culture astrocytes by a Ca^{2+} related A1/A2 adenosine receptor cooperation. *Eur. J. Neurosci.* 8, 1124–1131

b Peakman, M.C. and Hill, S.J. (1996) Adenosine A1 receptor-mediated inhibition of cyclic AMP accumulation in type-2 but not type-1 rat astrocytes. *Eur. J. Pharmacol.* 306, 281–289

c Peakman, M.C. and Hill, S.J. (1994) Adenosine A2B-receptor-mediated cyclic AMP accumulation in primary rat astrocytes. *Br. J. Pharmacol.* 111, 191–198

d Walz, W. *et al.* (1994) Extracellular ATP-induced currents in astrocytes: involvement of a cation channel. *J. Neurosci. Res.* 38, 12–18

e Loesch, A. and Burnstock, G. (1998) Electron-immunocytochemical localization of P2X1 receptors in the rat cerebellum. *Cell Tissue Res.* 294, 253–260

f Ballerini, P. *et al.* (1996) Rat astroglial P2Z (P2X7) receptors regulate intracellular calcium and purine release. *NeuroReport* 7, 2533–2537

g King, B.F. *et al.* (1996) P2 purinoceptors in rat cortical astrocytes: expression, calcium-imaging and signalling studies. *Neuroscience* 74, 1187–1196

h Fam, S.R. *et al.* (2000) P2Y(1) purinoceptor-mediated Ca^{2+} signaling and Ca^{2+} wave propagation in dorsal cord astrocytes. *J. Neurosci.* 20, 2800–2808

i Chen, W.C. and Chen, C.C. (1998) ATP-induced arachidonic acid release in cultured astrocytes is mediated by Gi protein coupled P2Y1 and P2Y2 receptors. *Glia* 22, 360–370

j Ho, C. *et al.* (1995) A novel P2-purinoceptor expressed by a subpopulation of astrocytes from the dorsal spinal cord of the rat. *Br. J. Pharmacol.* 116, 2909–2918

k Lenz, G. *et al.* (2000) P(2Y) purinoceptor subtypes recruit different MEK activators in astrocytes. *Br. J. Pharmacol.* 129, 927–936

l Kirischuk, S. *et al.* (1995) Activation of P2-purinoreceptors triggered Ca^{2+} release from InsP3-sensitive internal stores in mammalian oligodendrocytes. *J. Physiol.* 483, 41–57

m Lyons, S.A. *et al.* (1995) Schwann cell ATP-mediated calcium increases *in vitro* and *in situ* are dependent on contact with neurons. *Glia* 13, 27–38

n Mayer, C. *et al.* (1998) Differences in the sensitivity to purinergic stimulation of myelinating and non-myelinating Schwann cells in peripheral human and rat nerve. *Glia* 23, 374–382

o Ansselin, A.D. *et al.* (1997) Extracellular ATP increases intracellular calcium in cultured adult Schwann cells. *Neuroscience* 76, 947–955

p Grafe, P. *et al.* (1999) Confocal calcium imaging reveals an ionotropic P2 nucleotide receptor in the paranodal membrane of rat Schwann cells. *J. Physiol.* 515, 377–383

q Robitaille, R. (1995) Purinergic receptors and their activation by endogenous purines at perisynaptic glial cells of the frog neuromuscular junction. *J. Neurosci.* 15, 7121–7131

r Green, A.C. *et al.* (1997) ATP acting on P2Y receptors triggers calcium mobilization in Schwann cells at the neuroelectrocyte junction in skate. *Neuroscience* 80, 635–651

s Gebicke, P.J. *et al.* (1997) Both adenosine A1- and A2-receptors are required to stimulate microglial proliferation. *Neurochem. Int.* 29, 37–42

t Fiebich, B.L. *et al.* (1996) Cyclooxygenase-2 expression in rat microglia is induced by adenosine A2a-receptors. *Glia* 80, 635–651

u Ferrari, D. *et al.* (1996) Mouse microglial cells express a plasma membrane pore gated by extracellular ATP. *J. Immunol.* 156, 1531–1539

v Visentin, S. *et al.* (1999) Two different ionotropic receptors are activated by ATP in rat microglia. *J. Physiol.* 519, 723–736

w Dolphin, A.C. (1986) Calcium-dependent currents in cultured rat dorsal root ganglion neurones are inhibited by an adenosine analogue. *J. Physiol.* 373, 47–61

x Hu, H.Z. and Li, Z.W. (1997) Modulation by adenosine of GABA-activated current in rat dorsal root ganglion neurons. *J. Physiol.* 501, 67–75

y Toescu, E.C. *et al.* (1998) Long-term activation of capacitative Ca^{2+} entry in mouse microglial cells. *Neuroscience* 86, 925–935

z Grubb, B.D. and Evans, R.J. (1999) Characterization of cultured dorsal root ganglion neuron P2X receptors. *Eur. J. Neurosci.* 11, 149–154

aa Ueno, S. *et al.* (1999) Cell type-specific ATP-activated responses in rat dorsal root ganglion neurons. *Br. J. Pharmacol.* 126, 429–436

bb Vulchanova, L. *et al.* (1998) P2X3 is expressed by DRG neurons that terminate in inner lamina II. *Eur. J. Neurosci.* 10, 3470–3478

cc Svichar, N. *et al.* (1997) ATP induces Ca^{2+} release from IP3-sensitive Ca^{2+} stores exclusively in large DRG neurones. *NeuroReport* 8, 1555–1559

dd Ralevic, V. and Burnstock, G. (1998) Receptors for purines and pyrimidines. *Pharmacol. Rev.* 50, 413–492

might contribute to the neuronal loss associated with cerebral ischemia or traumatic brain injury[63]. Experimental infusion of ATP or P2 receptor agonists into the nucleus acumbens[64] or cerebral hemisphere[65] of rats suggests that purines might be a signal for the induction of malignant brain tumors. In these *in vivo*

experiments, ATP infusion resulted in increased astrocyte proliferation, formation of reactive astrocytes (GFAP-positive cells with multiple cellular processes) and, in several animals, the formation of gliomas. It has also been proposed that cell–cell communication involving extracellular ATP contributes to neurovascular changes responsible for the pain associated with migraine headaches[66]. Intercellular Ca^{2+}-waves in pia-arachnoid cells can be stimulated by mechanical stimulation. These waves, which propagate between pia-arachnoid cells and contiguous astrocytes, can be blocked by octanol or apyrase, indicating the involvement of gap-junction communication and extracellular ATP.

These pathophysiological and adaptive glial responses to the ATP that is released in association with cellular injury give some indication of the range of glial responses that can be regulated by ATP, and provide intriguing insight into neuron–glial functions that might be influenced by the activity-dependent release of ATP.

Directions for future research

Research on the glial cell of the PNS has shown that extracellular ATP is an important molecule in activity-dependent signaling between neurons and glia at the synapse and in non-synaptic regions. Research on glia of the CNS has shown the importance of ATP in astrocyte–astrocyte signaling and of neural impulse activity in regulating glial functions by the vesicular release of neurotransmitters. A future research area will be to determine whether ATP might also mediate neuron–glial signaling in the CNS at synaptic and extrasynaptic regions (Box 1). Could axon–oligodendrocyte signaling in the hippocampus involve ATP, in addition to excitatory neurotransmitters? Stimulus-dependent release of ATP has been measured from hippocampal slices[67] and purinergic receptors have been shown to be present on oligodendrocytes (Box 2). Could synaptic release of ATP stimulate calcium waves in astrocytes, much as has been shown for synaptic release of glutamate? Could ATP be an activity-dependent signal between retinal axons and astrocytes resulting in PDGF secretion and increased proliferation of OPC cells? Further research is needed to understand how ATP is released from non-synaptic regions of neurons in an activity-dependent manner. New functions might be regulated by ATP-dependent neuron–glial signaling in non-synaptic regions, because this does not relate directly to homeostasis of the extracellular environment. This might suggest possible medical implications of ATP-mediated neuron–glial signaling and possibly new pharmacological approaches to neurological disease and injury. In spite of, or perhaps because of, its universal familiarity to all students of biology, our current understanding of ATP only scratches the surface of what appear to be the many unique roles of this molecule outside the cell, including bridging the space between neurons and glia.

Selected references

1 Robitaille, R. (1995) Purinergic receptors and their activation by endogenous purines at perisynaptic glial cells of the frog neuromuscular junction. *J. Neurosci.* 15, 7121–7131
2 Stevens, B. and Fields, R.D. (2000) Response of Schwann cells to action potentials in development. *Science* 287, 2267–2271
3 Araque, A. *et al.* (1999) Tripartite synapses: glia, the unacknowledged partner. *Trends Neurosci.* 22, 208–215
4 LevRam, V. and Ellisman, M.H. (1995) Axonal activation-induced calcium transients in myelinating Schwann cells, sources and mechanisms. *J. Neurosci.* 15, 2628–2637
5 Bergles, D.E. *et al.* (2000) Glutamatergic synapses on oligodendrocyte precursor cells in the hippocampus. *Nature* 405, 187–191
6 Araque, A. *et al.* (2000) SNARE protein-dependent glutamate release from astrocytes. *J. Neurosci.*15, 666–673
7 Nedergaard, M. (1994) Direct signaling from astrocytes to neurons in cultures of mammalian brain cells. *Science* 263, 1768–1771
8 Parpura, V. *et al.* (1994) Glutamate-mediated astrocyte–neuron signaling. *Nature* 369, 744–747
9 Demerens, C. *et al.* (1996) Induction of myelination in the central nervous system by electrical activity. *Proc. Natl. Acad. Sci. U. S. A.* 93, 9887–9892
10 Stevens, B. *et al.* (1998) Control of myelination by specific patterns of neural impulses. *J. Neurosci.* 18, 9303–9311
11 Son, Y.J. *et al.* (1996) Schwann cells induce and guide sprouting and reinnervation of neuromuscular junctions. *Trends Neurosci.* 19, 280–285
12 Jahromi, B.S. *et al.* (1992) Transmitter release increases intracellular calcium in perisynaptic Schwann cells *in situ*. *Neuron* 8, 1069–1077
13 Reist, N.E. and Smith, S.J. (1992) Neurally evoked calcium transients in terminal Schwann cells at the neuromuscular junction. *Proc. Natl. Acad. Sci. U. S. A.* 89, 7625–7629
14 Georgiou, J. *et al.* (1994) Synaptic regulation of glial protein expression *in vivo*. *Neuron* 12, 443–455
15 Robitaille, R. (1998) Modulation of synaptic efficacy and synaptic depression by glial cells at the frog neuromuscular junction. *Neuron* 21, 847–855
16 Parpura, V. *et al.* (1995) Neuroligand-evoked calcium-dependent release of excitatory amino acids from Schwann cells. *J. Neurosci.* 15, 5831–5839
17 Ralevic, V. and Burnstock, G. (1998) Receptors for purines and pyrimidines. *Pharmacol. Rev.* 50, 413–492
18 Dubyak, G.R. and El-Moatassim, C. (1993) Signal transduction via P2-purinergic receptors for extracellular ATP and other nucleotides. *Am. J. Physiol.* 265, C577–C606
19 Gousley, G.D. (2000) Physiological effects of extracellular nucleotides in the inner ear. *Clin. Exp. Pharmacol. Physiol.* 27, 575–580
20 Osipchuk, Y. and Cahalan, M. (1992) Cell-to-cell spread of calcium signals mediated by ATP receptors in mast cells. *Nature* 359, 241–244
21 Guthrie, P.B. *et al.* (1999) ATP release from astrocytes mediates glial calcium waves. *J. Neurosci.* 19, 520–528
22 Cornell-Bell, A.H. *et al.* (1990) Glutamate induces calcium waves in cultured astrocytes: long-range glial signaling. *Science* 247, 470–473
23 Fam, S.R. *et al.* (2000) P2Y1 purinoceptor-mediated Ca^{2+} signaling and Ca^{2+} wave propogation in dorsal spinal astrocytes. *J. Neurosci.* 20, 2800–2808
24 Newman, E.A. and Zahs, K.R. (1997) Calcium waves in retinal glial cells. *Science* 275, 844–847
25 Newman, E.A. and Zahs, K.R. (1998) Modulation of neuronal activity by glial cells in the retina. *J. Neurosci.* 18, 4022–4028
26 Jeftinija, S.D. and Jeftinija, K.V. (1998) ATP stimulates release of excitatory amino acids from cultured Schwann cells. *Neuroscience* 82, 927–934
27 Edwards, F.A. *et al.* (1992) ATP receptor-mediated synaptic currents in the central nervous system. *Nature* 359, 144–147
28 Gu, J.G. and MacDermott, A.B. (1997) Activation of ATP P2X receptors elicits glutamate release from sensory neuron synapses. *Nature* 389, 749–753
29 Zimmermann, H. (1994) Signaling via ATP in the nervous system. *Trends Neurosci.* 17, 420–426
30 Jo, Y-H. and Schlichter, R. (1999) Synaptic corelease of ATP and GABA in cultured spinal neurons. *Nat. Neurosci.* 2, 241–245
31 Bo, X. and Brunstock, G. (1994) Distribution of [3H] α, beta-methylene ATP binding sites in rat brain and spinal cord. *NeuroReport* 5, 1601–1604
32 von Kugelgen, I. *et al.* (1999) Role of action potentials and calcium influx in ATP- and UDP-induced noradrenaline release from rat cultured sympathetic neurones. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 359, 360–369
33 Silinsky, E.M. *et al.* (1999) Quantal ATP release from motor nerve endings and its role in neurally mediated depression. *Prog. Brain Res.* 120, 145–158
34 Zimmermann, H. (1998) New insights into molecular structure and function of ectonucleosidases in the nervous system. *Neurochem. Int.* 32, 421–425
35 Santos, P.F. *et al.* (1999) Characterization of ATP release from cultures enriched in cholinergic amacrine-like neurons. *J. Neurobiol.* 41, 340–348
36 Inoue, K.I. (1998) The functions of ATP receptors in the hippocampus. *Pharmacol. Res.* 38, 323–331
37 Wachtler, J. *et al.* (1998) Activity-dependent intracellular calcium transients in unmyelinated nerve fibers of the isolated adult rat vagus nerve. *Pflügers Arch. Eur. J. Physiol.* 435, 678–686

38 Kriegler, S. and Chiu, S.Y. (1993) Calcium signaling of glial cells along mammalian axons. *J. Neurosci.*13, 4229–4245
39 Kuperman, A.S. *et al.* (1964) Release of adenine nucleotides from nerve axons. *Nature* 204, 1000–1001
40 Weinneich, D. and Hammerschlag, R. (1975) Nerve impulse-enhanced release of amino acids from non-synaptic regions of peripheral and central nerve trunks of bullfrog. *Brain Res.* 84, 137–142
41 Reisin, I.L. *et al.* (1994) The cystic fibrosis transmembrane conductance regulator is a dual ATP and chloride channel. *J. Biol. Chem.* 269, 20584–20591
42 Abraham, E.H. *et al.* (1993) The multidrug resistance (mdr1) gene product functions as an ATP channel. *Proc. Natl. Acad. Sci. U. S. A.* 90, 312–316
43 Reddy, M.M. *et al.* (1996) Failure of the cystic fibrosis transmembrane conductance regulator to conduct ATP. *Science* 271, 1876–1879
44 Grygorczyk, R. and Hanrahan, J.W. (1997) CFTR-dependent ATP release from epithelial cells triggered by mechanical stimuli. *Am. J. Physiol.* 272, C1058–C1066
45 Jiang, Q. *et al.* (1998) Cystic fibrosis transmembrane conductance regulator-associated ATP release is controlled by a chloride sensor. *J. Cell Biol.* 143, 645–657
46 Cotrina, M.L. *et al.* (1998) Connexins regulate calcium signaling by controlling ATP release. *Proc. Natl. Acad. Sci. U. S. A.* 95, 15735–15740
47 Trexler, E.B. *et al.* (1996) Voltage gating and permeation in a gap junction hemichannel. *Proc. Natl. Acad. Sci. U. S. A.* 93, 5836–5841
48 Li, H. *et al.* (1996) Properties and regulation of gap junctional hemichannels in the plasma membranes of cultured cells. *J. Cell Biol.* 134, 1019–1030
49 Cotrina, M.L. *et al.* (2000) ATP-mediated glia signaling. *J. Neurosci.* 20, 2835–2844
50 Kandler, K. and Katz, L.C. (1995) Neuronal coupling and uncoupling in the developing nervous system. *Curr. Opin. Neurobiol.* 5, 98–105
51 Mirsky, R. and Jessen, K.R. (1999) The neurobiology of Schwann cells. *Brain Pathol.* 9, 293–311
52 Fitzgerald, M. (1987) Spontaneous and evoked activity of fetal primary afferents *in vivo*. *Nature* 326, 603–605
53 Wang, D.J. (1991) Multiple signal transduction pathways lead to extracellular ATP-stimulated mitogenesis in mammalian cells: I. Involvement of protein kinase C-dependent and independent pathways. *J. Cell Physiol.* 146, 473–482
54 Neary, J.T. *et al.* (1999) Mitogenic signaling by ATP/P2Y purinergic receptors in astrocytes: involvement of a calcium-independent protein kinase C, extracellular signal-regulated protein kinase pathway distinct from the phosphatidylinositol-specific phospholipase C/calcium pathway. *J. Neurosci.* 19, 4211–4220
55 Gallo, V. *et al.* (1996) Oligodendrocyte progenitor cell proliferation and lineage progression are regulated by glutamate receptor-mediated K^+ channel block. *J. Neurosci.* 16, 2659–2670
56 Barres, B.A. and Raff, M.C. (1993) Proliferation of oligodendrocyte precursor cells depends on electrical activity in axons. *Nature* 361, 258–260
57 Grafe, P. *et al.* (1999) Confocal calcium imaging reveals an ionotropic P2 nucleotide receptor in the paranodal membrane of rat Schwann cells. *J. Physiol.* 515, 377–383
58 Mayer, C. *et al.* (1998) Differences in the sensitivity to purinergic stimulation of myelinating and non-myelinating Schwann cells in peripheral human and rat nerve. *Glia* 23, 374–382
59 Green, A.C. *et al.* (1997) ATP acting on P2Y receptors triggers calcium mobilization in Schwann cells at the neuroelectrocyte junction in skate. *Neuroscience* 80, 635–651
60 Fulton, B.P. (1995) Gap junctions in the developing nervous system. *Perspect. Dev. Neurobiol.* 2, 327–334
61 Rathbone, M.P. *et al.* (1998) The trophic effects of purines and purinergic signaling in pathologic reactions of astrocytes. *Alzheimer Dis. Assoc. Disord.* 12 (Suppl. 2), S36–S45
62 Illes, P. *et al.* (1996) Molecular mechanisms of microglial activation. B. Voltage- and purinoceptor-operated channels in microglia. *Neurochem. Int.* 29, 13–24
63 Stella, N. *et al.* (1997) Interleukin-1 enhances the ATP-evoked release of arachidonic acid from mouse astrocytes. *J. Neurosci.* 17, 2939–2946
64 Franke, H. *et al.* (1999) P2 receptor-mediated proliferative effects on astrocytes *in vivo*. *Glia* 28, 190–200
65 Hindley, S. *et al.* (1994) Stimulation of reactive astrogliosis *in vivo* by extracellular adenosine diphosphate or an adenosine A2 receptor agonist. *J. Neurosci. Res.* 38, 399–406
66 Grafstein, B. *et al.* (2000) Meningeal cells can communicate with astrocytes by calcium signaling. *Ann. Neurol.* 47, 18–25
67 Wieraszko, A. *et al.* (1989) Stimulation-dependent release of adenosine triphosphate from hippocampal slices. *Brain Res.* 485, 244–250
68 Gilmore, S.A. and Sims, T.J. (1995) Glial cells and regeneration in the peripheral nervous system. In *Neuroglia* (H. Kettenmann and B.R. Ransom, eds) Oxford University Press

The evolution of cortical development. An hypothesis based on the role of the Reelin signaling pathway

Isabelle Bar, Catherine Lambert de Rouvroit and André M. Goffinet

Expression of the genes encoding Reelin and Dab1 during cortical development in turtle, lizard, chick and mammals correlates with architectonic patterns. In all species, Reelin is secreted by marginal zone cells, whereas Dab1, which mediates the response to Reelin, is synthesized by cortical plate neurons. This pattern was presumably present in stem amniotes. In mammals, the cortical plate is radially organized and develops from inside to outside, these features depend on amplification of reelin synthesis in the marginal zone. In lizards, the cortical plate develops from outside to inside, similar to other non-mammals, but is radially organized, with an additional layer of Reelin added in the subcortex. Thus, the Reelin pathway played a key role in cortical architectonic evolution in mammalian and squamate lineages.

Trends Neurosci. (2000) 23, 633–638

GENES THAT CONTROL brain development and growth are obvious targets of the evolutionary process[1]. Unfortunately, the study of the evolution of development is severely hampered by the poor fossilization of immature individuals and the lack of fossilization of brain tissue. Our understanding of brain evolution is thus based on inference from comparative analyses of the neuroanatomy (including gene expression patterns) of living organisms. Similarly, the evolution of brain development can be

Isabelle Bar, Catherine Lambert de Rouvroit and André M. Goffinet are at the Neurobiology Unit, University of Namur, School of Medicine, 61 rue de Bruxelles, B-5000 Namur, Belgium.

 PII: S0166-2236(00)01675-1