

Severe Convulsions and Dysmyelination in Both Jimpy and *Cx32/47*^{-/-} Mice may Associate Astrocytic L-Channel Function with Myelination and Oligodendrocytic Connexins with Internodal K_v Channels

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Abstract The Jimpy mouse illustrates the importance of interactions between astrocytes and oligodendrocytes. It has a mutation in *Plp* coding for proteolipid protein and DM20. Its behavior is normal at birth but from the age of ~2 weeks it shows severe convulsions associated with oligodendrocyte/myelination deficits and early death. A normally occurring increase in oxygen consumption by highly elevated K⁺ concentrations is absent in Jimpy brain slices and cultured astrocytes, reflecting that *Plp* at early embryonic stages affects common precursors as also shown by the ability of conditioned medium from normal astrocytes to counteract histological abnormalities. This metabolic response is now known to reflect opening of L-channels for Ca²⁺. The resulting deficiency in Ca²⁺ entry has many consequences, including lack of K⁺-stimulated glycogenolysis and release of gliotransmitter ATP. Lack of purinergic stimulation compromises oligodendrocyte survival and myelination and affects connexins and K⁺ channels. Mice lacking the oligodendrocytic connexins Cx32 and 47 show similar neurological dysfunction as Jimpy. This possibly reflects that K⁺ released by internodal axonal K_v channels is transported underneath a loosened myelin sheath instead of reaching the extracellular space via connexin-mediated transport to oligodendrocytes, followed by release and

astrocytic Na⁺,K⁺-ATPase-driven uptake with subsequent Kir4.1-facilitated release and neuronal uptake.

Keywords Astrocytic L-channels for Ca²⁺ · Astrocytic-oligodendrocytic interaction · *Cx32/47*^{-/-} convulsions/dysmyelination · Dysmyelination · Glial connexins · Gliotransmitter ATP · Jimpy · Leukemia inhibitory factor · Proteolipid protein · White matter K⁺ homeostasis

Introduction

Amaral et al. [1] encouraged the scientific community to unravel oligodendrocyte interaction with astrocytes and neurons. In response to this challenge we have re-interpreted almost 40 year-old observations in developing Jimpy mice. These animals, which have a gene defect in the *Plp* gene coding for proteolipid protein (PLP) synthesis, appear to be normal at birth but are deficient in myelination, show greatly increased oligodendrocytic cell death and die by the age of 30 days, often following seizures. Skoff [2] suggested that they also suffer from astrocytic abnormalities. Our group [3–5] has found that astrocytes cultured from newborn Jimpy mice lack a specific astrocyte-characteristic feature, i.e., stimulation of oxidative metabolism by exposure to highly elevated (60 mM) extracellular concentrations of the potassium ion, K⁺ ([K⁺]_o). However other studied functions, including K⁺ and glutamate uptake, were normal. At that time it was unknown which activities are reflected by stimulation of astrocytic O₂ uptake by [K⁺]_o exceeding 15 mM. However, it is now well established that it is a metabolic response to opening of L-channels for Ca²⁺. In addition more information about the Jimpy mouse has become available, especially regarding rescue of cultured Jimpy oligodendrocytes by astrocyte-conditioned

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medium. Relevant aspects of these newer studies on Jimpy mice will initially be reviewed (“[The Jimpy Mouse](#)” section). This will be followed by a summary of our previous published and non-published (except for G. Chaban’s thesis [4]) findings in cultured Jimpy astrocytes (“[Increased \$K^+\$ Concentrations Fail to Increase Oxygen Consumption in Jimpy Astrocytes but Other Functions are Normal](#)” section), key points of current knowledge about consequences of L-channel activation in astrocytes (“ [\$O_2\$ Uptake Stimulation by High \$\[K^+\]_o\$ Reflects L-Channel Mediated \$Ca^{2+}\$ Uptake Stimulating Glycogenolysis, ATP Release and \$NKCC1\$](#) ” section) and how failing glycogenolysis-dependent ATP release might affect oligodendrocyte maturation and myelination (“[Release of Transmitter ATP from Axons and Astrocytes Promotes Oligodendrocyte Development and Myelination](#)” section). It probably also affects formation of connexins and gap junctions, and “[Knockout of Oligodendrocytic Connexins Causes Jimpy-like Symptoms](#)” section describes oligodendrocytic and astrocytic connexins and how knockout of two oligodendrocytic connexins leads to virtually similar neurological dysfunction as that seen in Jimpy. Finally a hypothesis is presented how demyelination or connexin knockout may affect K^+ homeostasis, in the latter case by compromising K^+ transport from K^+ after exit from the axon via internodal K_v channels (“[Is \$K^+\$ Homeostasis Compromised in JIMPY and \$Cx32/47\$ Knockouts?](#)” section).

The Jimpy Mouse

Studies In Vivo

The Jimpy mouse has an X-linked recessive mutation in *Plp*, the gene coding for PLP and its shorter splice variant DM20 leading to dysmyelination and seizures. The number of oligodendrocytes in Jimpy is normal at birth and early postnatally, but oligodendrocytes fail to fully differentiate in the central nervous system and the mice die soon after the onset of a rudimentary myelination [6–9]. Neurological symptoms have repeatedly been reported to begin between postnatal days 9 and 15 [10–12]. Nave et al. [6] described a body tremor before motor activity, which appears about postnatal day 11, and 1 week later changes into convulsions. This is consistent with other authors [10, 11, 13, 14] reporting the first seizures between postnatal days 15 and 21. Most authors agree that death occurs around the age of 1 month but survival for up to 50 days has been reported [15]. The seizures can be elicited by handling of the animals, and Fig. 1 shows individual frames from a cinematographic recording of seizure development and recovery [4].

The neurological symptoms appear at an ontogenetic stage when cerebral and cerebellar myelination is underway

in normal mice [16]. They are associated with a very pronounced deficit in myelin formation in the central nervous system (CNS) combined with a drastic decrease in the number of oligodendrocytes [17–19]. Axonal diameters are reduced [20], whereas there is astroglial hypertrophy with an increased number of cell processes, compromising interaction with oligodendrocytes and axons [2, 18, 21]. In the optic nerve of 21-day-old Jimpy mice the ultrastructural appearance of axons and astrocyte cell bodies seems normal, some oligodendrocytes also look normal but others are filled with lipid inclusions, an indication of dying cells, and myelin is almost absent [22] (Fig. 2). However, impulse propagation occurs since the mice are not blind. More myelinated axons have been observed in spinal cord, but the myelin sheath is morphologically abnormal, with reduced tight contact with the axon [23, 24].

Myelination in the peripheral nervous system is normal, in agreement with differences between functional characteristics of oligodendrocytes and Schwann cells and of myelin in gray and white matter in normal animals [25, 26]. Normally the potential for regeneration of myelin after demyelination is also different, since white matter is able to regain full functionality after demyelination while cortical gray matter lesions cause permanently altered network function [27].

In the light of the severe symptomatology in Jimpy it is remarkable that several findings indicate that PLP or DM20 are not necessary for myelin formation. Even in the absence of *Plp*, mice can survive without gross behavioral effects in their first year and they have a fairly normal life span [28, 29], although the compaction of their myelin sheath is abnormal [30]. Different more or less severe neurological problems occur in humans lacking proteolipid protein [31]. For the understanding of the neurological defects in both mouse and man it is very important that PLP plays additional roles in neural development beyond functioning as a structural component of myelin [32]. Replacement in mice optic nerve of PLP with the peripheral nerve myelin protein, P_0 , leads to mitochondrial pathology and degeneration in the axoplasm of 1-month old mice, which are prominent in the juxtaparanodal region and associated with decreased ATP content [33]. This is different from the normal mitochondrial function in Jimpy astrocytes shown by Best et al. [21]. Moreover, widespread expression of transcripts encoding PLP and DM20 is found during embryonic and early postnatal CNS development, and specificity to the oligodendrocyte cell lineage develops only at later postnatal stages during myelination [34, 35]. PLP is expressed at least 1 week before myelination and its injection before postnatal day 2 leads to secretion of a factor that increases the proliferation of not only oligodendrocyte but also astrocyte lineage cells [36]. In its absence some small-diameter axons lack a compact sheath or show delayed myelination,

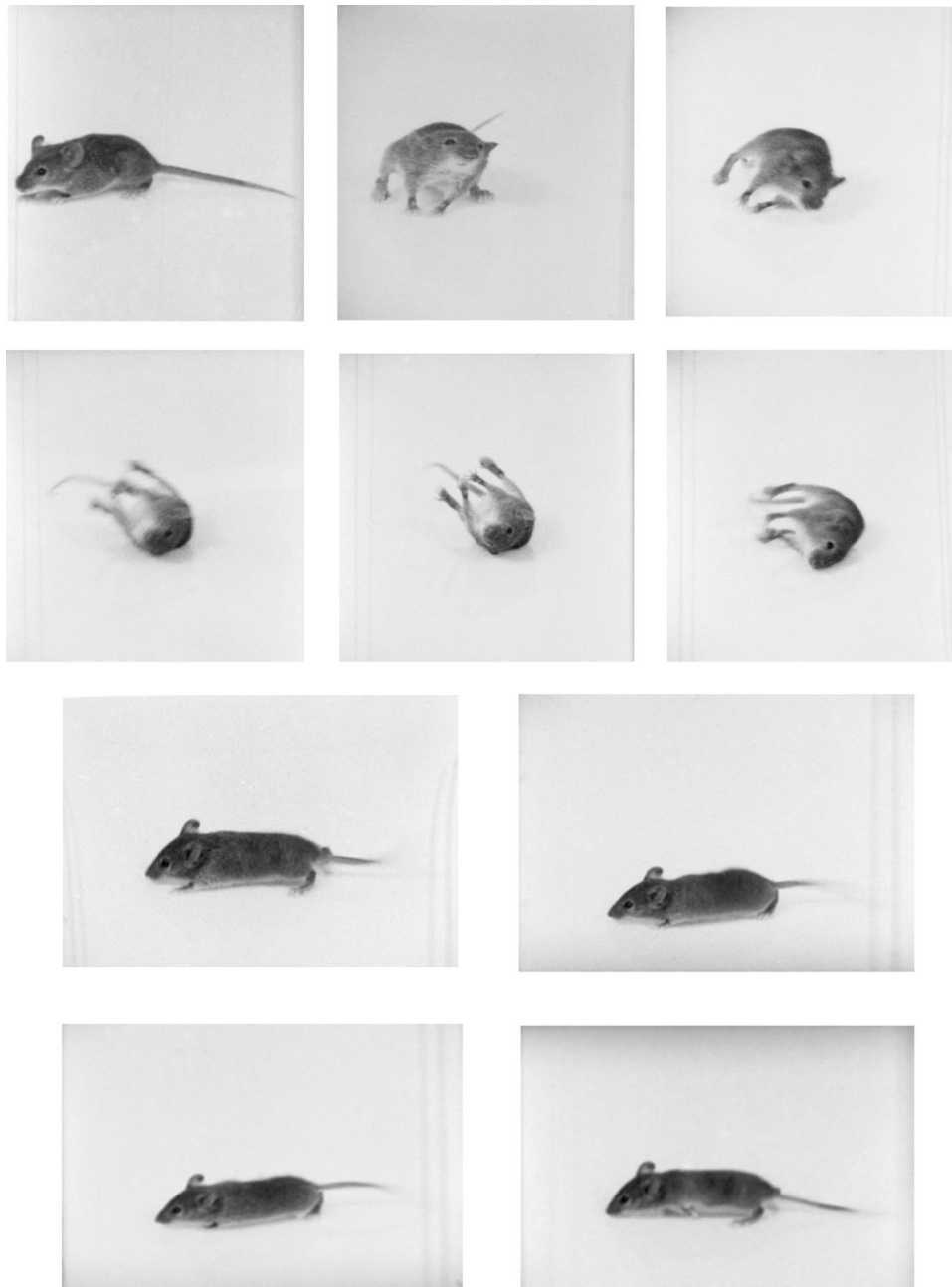


Fig. 1 Selected pictures illustrating a 22 s seizure in a 20-day-old Jimpy mouse. From Chaban, 1980 [4]. For a movie of a seizure see website link: https://drive.google.com/file/d/0ByRmcg3mTqsedGRfNFNnN1M4SWs/view?usp=drive_web

suggesting that PLP/DM20 is involved in early stages of axon-oligodendrocyte interaction and wrapping of the axon [37]. Jimpy mice show cell cycle abnormalities in both oligodendrocytes and astrocytes and there is a large increase in microglia [34, 38, 39].

In spite of the abnormalities in animals with lack of PLP and a null mutation of its gene they behave much more normally than animals with extra *Plp* gene

copies or missense mutations [40]. Duplication of *Plp* in rodents disable many functions, and in humans PLP overexpression is the most common etiology of the debilitating Pelizaeus-Merzbacher disease [32, 41]. The overexpression is associated with mitochondrial dysfunction [40], whereas oligodendrocytes cultured from brains of Jimpy mice have normal mitochondrial function [21, 42].

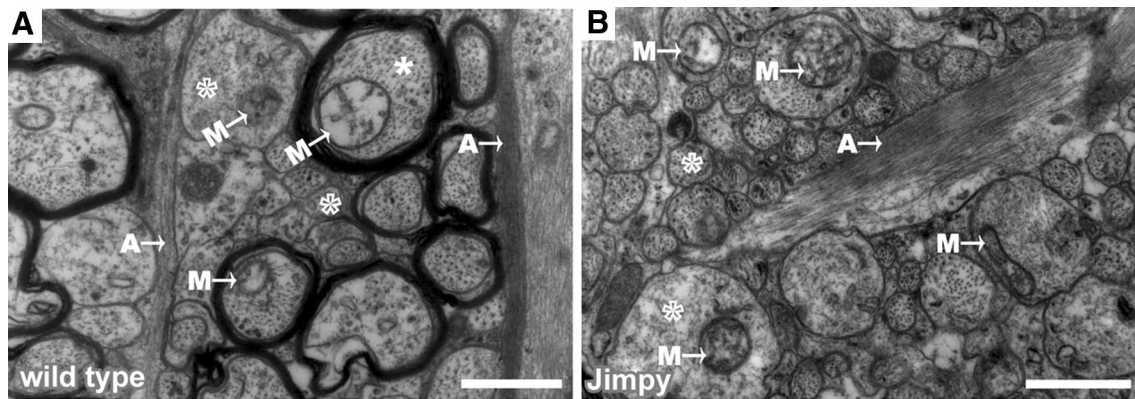


Fig. 2 Electron micrographs of optic nerve cross sections from wild-type mouse (a) and Jimpy mutant that does not carry the Tabby mutation (b) shown at high magnification (scale bar 1 μ m). The ultrastructural appearance of the axons in the Jimpy mouse seems normal,

including the appearance of their mitochondria (M). However, in contrast to the wild type mouse most axons are not myelinated. Astrocytes (A) show normal morphology. From [22]

Cultured Oligodendrocytes

Most if not all characteristics of Jimpy oligodendrocytes are maintained in brain oligodendrocytes cultured in conventional ways, including protein supplementation and a low glucose concentration [43–45]. Cell numbers are normal in the cultures and the cells stain for typical glycolipids [9, 43]. However, they fail to develop extensive process networks or large myelin-like membrane sheets or substantial amounts of myelin proteins [15, 45], and only little myelin basic protein (MBP) can be detected [46, 47]. These deficiencies are ameliorated when Jimpy oligodendrocytes are grown in medium conditioned by normal astrocytes or by DM20 transfected NIH3T3 cells. When grown in the conditioned media the oligodendrocytes produce myelin-like membranes and MBP and some cells express PLP [8, 43, 45], which is of the Jimpy type [44]. This is consistent with the relative lack of adverse effects of PLP deletion seen in vivo, although Williams and Gard [48] using less traditional procedures for culturing of both oligodendrocytes and astrocytes did not replicate the beneficial effect of conditioned media. In addition cultured Jimpy oligodendrocytes as well as oligodendrocytes isolated directly from Jimpy brain show an approximately doubling of free cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) [45]. This abnormality is not counteracted by conditioned medium from DM20 transfected NIH3T3 cells and astrocytic $[Ca^{2+}]_i$ is not affected.

Increased K^+ Concentrations Fail to Increase Oxygen Consumption in Jimpy Astrocytes but Other Functions are Normal

Keen et al. [49] found that the stimulation of oxygen uptake which normally occurs as a result of exposure to high

concentrations of potassium ion (K^+) is almost absent in brain slices from Jimpy mice. We confirmed this observation [3, 4] and showed that the effect could be reproduced in cultured Jimpy astrocytes which were compared with cultures of their non-affected littermates (Fig. 3). The cells were prepared from new-born animals without use of enzymes and grown in uncoated 30-mm Falcon Petri dishes in a medium very similar to a low glucose (6.5 mM), bicarbonate-buffered Dulbecco's medium with addition of horse serum and from the age of 2 weeks also dibutyryl cyclic AMP (dBcAMP) [50, 51]. This compound substitutes for the noradrenergic stimulation the cells would have received in vivo. The cultures were at least 3 week-old when used and we never use subculturing. Such cultures from normal mice are with a few exceptions similar to astrocytes freshly isolated from the brain [52]. Ninety-five percent of the cells stain for the astrocytic markers GFAP and glutamine synthetase, neurons are absent, an occasional type 2 astrocyte is rarely seen and macrophages constitute 3% [50, 51]. Both the littermates and Jimpy astrocytes show a pronounced extension of processes in response to treatment with dBcAMP [4] (Fig. 4). This is in contrast to oligodendrocytes from Jimpy mice which do not respond to dBcAMP with process extension, although normal oligodendrocyte cultures do extend processes, although of different morphology than the astrocytic processes [53].

Oxygen uptakes were measured as previously described [54] in at least 4-week-old cultures by aid of an oxygen electrode in the closed flask filled with medium so that no air space functioning as an oxygen reservoir was present. The flask was closed with its usual stopper through which two small holes had been pierced, and placed in a water bath at 37 °C. A needle tip oxygen microelectrode and a reference electrode were inserted through the holes which subsequently were closed with melted beeswax. The

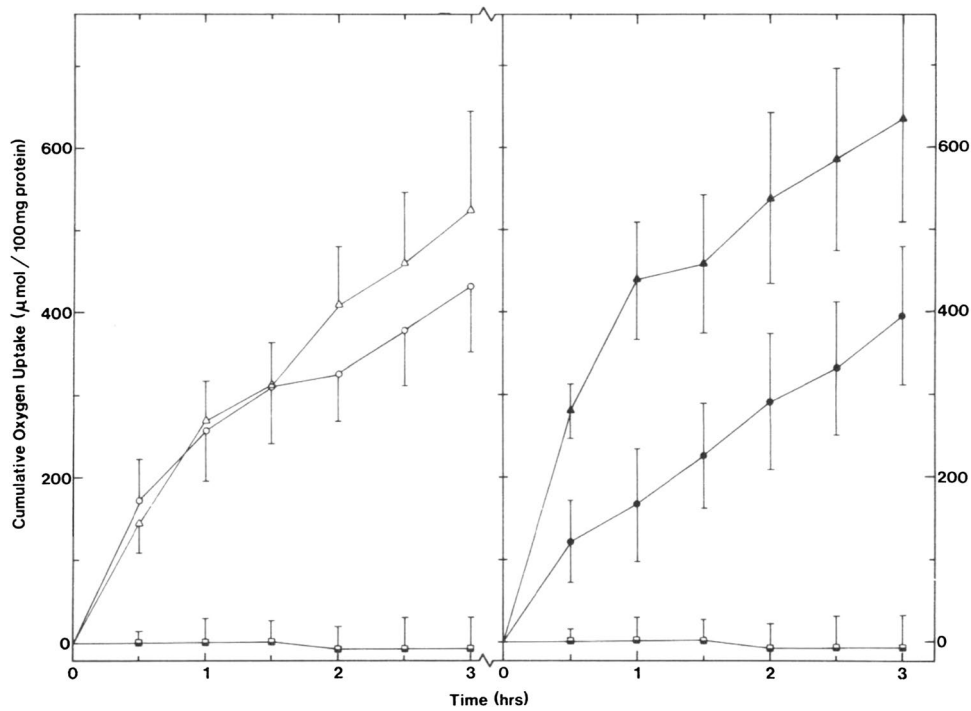
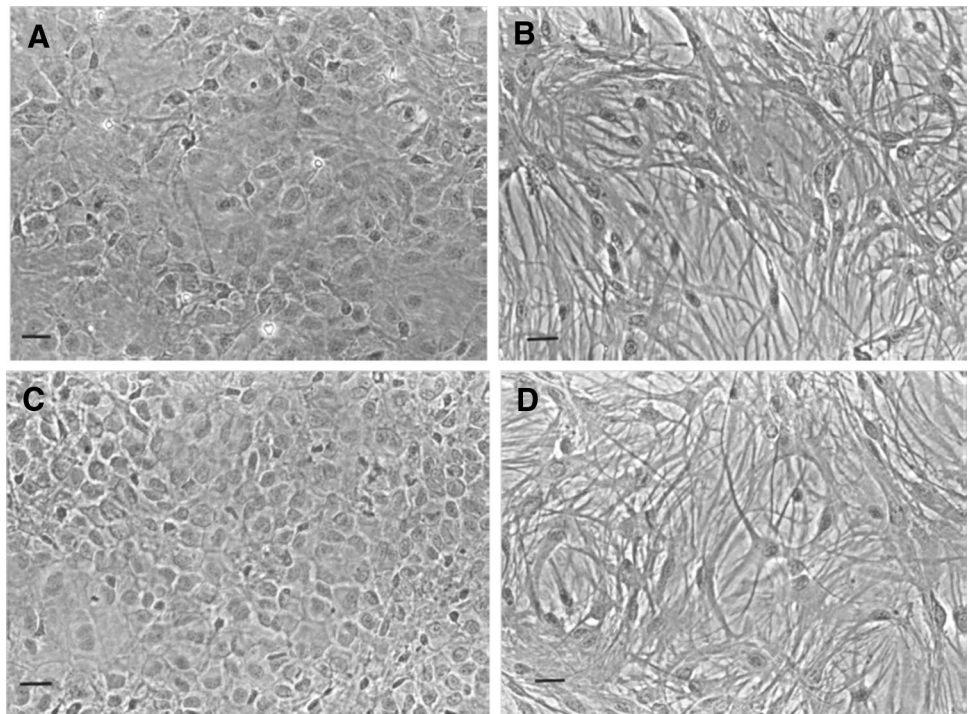


Fig. 3 Cumulative oxygen uptake by mouse astrocytes from Jimpy animals (*left side*) and normal littermates (*right side*) during 3 h of incubation in a HEPES-buffered modified MEM containing 5 mM K⁺ (*circles*) or 55 mM K⁺ (*triangles*). Results are means of 5–6 experiments, using an O₂ electrode inserted into a culture flask filled completely with medium. S.E.M. are indicated by vertical bars, and the results are expressed per 100 mg protein, i.e., approximately 1 g wet

weight. Note the initial stimulation by high K⁺ in control but not in Jimpy cultures. The lowest curve shows the lack of apparent respiration by flasks with medium but no cells (n=4). Another electrode did show a small drift which was subtracted from the respiratory rates. For graphical reasons, some S.E.M. values are indicated in one direction only. From [3]

Fig. 4 Phase contrast micrographs of three-week old astrocyte cultures grown as described in the text and but without (**a, c**) or with (**b, d**) dBcAMP addition during the third week. The medium was removed and the cultures fixed with absolute methanol. **a, b** are from non-Jimpy littermates and **c, d** from Jimpy mice. Note the homogeneity of the cultures and the similarity between Jimpy mice and their littermates and that the addition of dBcAMP in both cases causes a morphological differentiation from closely packed cells of epithelial appearance to astrocyte-like cells expressing a dense network of processes. This morphological effect of dBcAMP is accompanied by functional differentiation as shown by stimulation of the Na⁺, K⁺-ATPase activity (Table 1). The *bar* represents 30 μm. From [4]



signal from the electrodes was amplified and recorded on a Grass recorder, and respiratory rates were calculated. The medium was prepared with 20 mM Hepes (pH 7.3) instead of bicarbonate (which disturbs polarographic measurements due to deposit on the electrode). However, previous experiments with brain slices have shown little difference between oxidative metabolism in media with and without bicarbonate/CO₂ [55, 56].

Since the Jimpy mutation is recessive, only half of the offspring will express the Jimpy gene (*jp*) but new-born animals will not show any signs of the condition. A marker gene for Tabby, *ta* also known as *eda*, localized on the same chromosome as *jp*, was therefore used to identify new-born Jimpy animals. A stock of *tajp* females was obtained from the Jackson Laboratories, Bar Harbor, Maine and mated with normal C-57 BL males. At the day of birth, the new-born males were separated into groups of Tabby and of non-Tabby animals, based on the presence of one supra-orbital vibrissa and no postorbital vibrissa or of two supra-orbital vibrissae and one postorbital vibrissa, respectively [57]. Separate batches of cultures were prepared from the Tabby (and Jimpy) and the non-Tabby (and non-Jimpy) animals. Both types of cultures were prepared and grown in the usual manner. No detailed morphological/histochemical examination was performed, but the overall appearance of the cultures was similar to those grown from normal animals [4].

Rates of Oxygen Uptake During Exposure to 5 and to 55 mM K⁺

In the medium with 5 mM K⁺ the rates of oxygen uptake (indicated by the steepness of the graph showing cumulative oxygen consumption) by cells from Jimpy mice (Fig. 3, left side) was initially similar to previous observations in normal cultured astrocytes [54]. The rate of oxygen uptake was fastest (i.e. the slope steepest) at the beginning of the experiment (~250 μmol/hour/100 mg protein) and subsequently showed a small decline, again similar to results from normal astrocytes [54]. In the normal littermates (Fig. 3, right side) the rate of oxygen uptake appeared initially to be lower than in the Jimpy animals (and than that previously observed in normal mice [54]) but the difference was not statistically significant, and since the subsequent reduction in respiratory rate was less than in the Jimpy mice the cumulative oxygen uptakes were identical after 3 h in the Jimpy mice and their littermates. In the presence of the high concentration of K⁺, added to the normal medium, the rate of oxygen uptake by astrocytes from normal littermates (Fig. 3, right side) was initially almost doubled, a statistically significant difference ($P < 0.05$), but this increase was only maintained for about 1 h. This course is similar

to previous observations in normal cultured astrocytes [54] and in brain slices [55], where K⁺-induced stimulation of metabolism is known to be mainly glial [58]. In contrast, no initial increase was found in the cultures from Jimpy mice (Fig. 3, left side). There was also no subsequent marked decline, so in the long run (3 h) the cumulative oxygen uptake was only slightly lower than in normal littermates in a medium with 5 mM K⁺. Thus, at normal extracellular K⁺ ([K⁺]_o) oxidative metabolism is almost identical in Jimpy and non-Jimpy cultures, confirming the normal mitochondrial function in Jimpy mice [21, 22, 42], but the normal transient stimulation by excess [K⁺]_o is absent.

K⁺ Uptake Rate as a Function of [K⁺]_o and Na⁺, K⁺-ATPase Activity

In spite of the absence of any stimulation of O₂ uptake by highly elevated [K⁺]_o Fig. 5 shows that the uptake of K⁺ was similar in Jimpy and non-Jimpy cultures across the entire concentration range between 2.5 and 60 mM [K⁺]_o [4, 5]. As will be discussed in more detail in “O₂ Uptake Stimulation by High [K⁺]_o Reflects L-Channel Mediated Ca²⁺ Uptake Stimulating Glycogenolysis, ATP Release and NKCC1” section, minor increases in [K⁺]_o (up to 10 mM) selectively stimulate the Na⁺,K⁺-ATPase, whereas larger increases in addition stimulate NKCC1 [59, 60], a co-transporter of Na⁺, K⁺, 2 Cl⁻ and water [61–64]. The Na⁺,K⁺-ATPase activity was identical in Jimpy and control astrocytes, and in both it was higher in cultures treated with dBcAMP than in untreated cultures (Table 1). The lack of stimulation of O₂ uptake by highly elevated [K⁺]_o indicates that NKCC1 in Jimpy mice is not activated by elevated [K⁺]_o (see “O₂ Uptake Stimulation by High [K⁺]_o Reflects L-Channel Mediated Ca²⁺ Uptake Stimulating Glycogenolysis, ATP Release and NKCC1” section) but this transporter is also stimulated by extracellular hypertonicity [65–70]. Since the high-K⁺ medium was prepared by addition of excess KCl to a normal, isotonic medium NKCC1 stimulation by hypertonicity probably explains the normal K⁺ uptake at highly elevated [K⁺]_o.

Table 1 Na⁺, K⁺-ATPase activity in cultured astrocytes from Jimpy mice and normal littermates, measured as nmol liberated phosphate/hr per mg protein

dBcAMP during culturing	Jimpy	Normal littermates
+	3.20 ± 0.23 (n=2)	3.06 ± 0.01 (n=2)
-	4.33 ± 0.02 (n=2)	4.67 ± 0.21 (n=2)

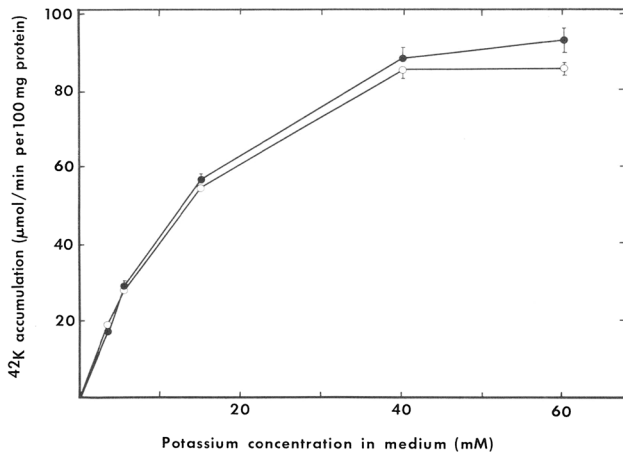
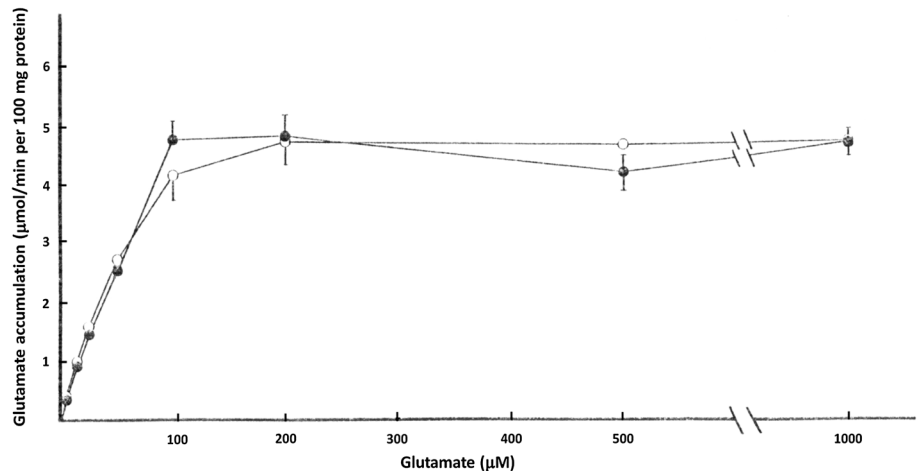


Fig. 5 Rate of K⁺ uptake (µmol/min per 100 mg protein), measured with ⁴²K during a 30 s. period, into cultured astrocytes from Jimpy animals (*open circles*) or normal littermates (*filled circles*) as a function of the external K⁺ concentration. Since the cultures had been depleted of K⁺ by incubation in ice-cold K⁺ free medium before the uptake measurement in order to prevent homoexchange with intracellular K⁺ the uptakes represent active uptake rates, which up to an extracellular K⁺ concentration of 10 mM are mediated exclusively by the Na⁺, K⁺-ATPase and at higher K⁺ concentrations jointly by the Na⁺, K⁺-ATPase and NKCC1 [59]. Since high K⁺ concentrations are unlikely to stimulate NKCC1 in the Jimpy mice, it is probably stimulated by hypertonicity in the K⁺-rich media, which were made by addition of KCl to the isotonic medium. Results are means ± SEM of 3–5 individual experiments. For further details, including statistical tests, see [4]

Glutamate and GABA Uptake, Glutaminase Activity and Contents of Glutamate and Glutamine

Uptake of glutamate (Fig. 6) was identical in Jimpy and control cultures [4] and similar to previously measured uptakes in normal astrocytes [71]. In both types of cultures it was higher at [K⁺]_o of 3–20 mM than in the absence of K⁺ [4], suggesting similar K⁺ permeability and pH

Fig. 6 Rate of glutamate uptake (µmol/min per 100 mg protein), measured with L-[¹⁴C] glutamate during a 2 min. period, into cultured astrocytes from Jimpy animals (*open circles*) or normal littermates (*filled circles*) as a function of the external glutamate concentration. Since glutamate homoexchange is insignificant [71] no preincubation in glutamate-free medium was performed. Results are means ± SEM of 4–16 individual experiments. For further details, including statistical tests, see [4]



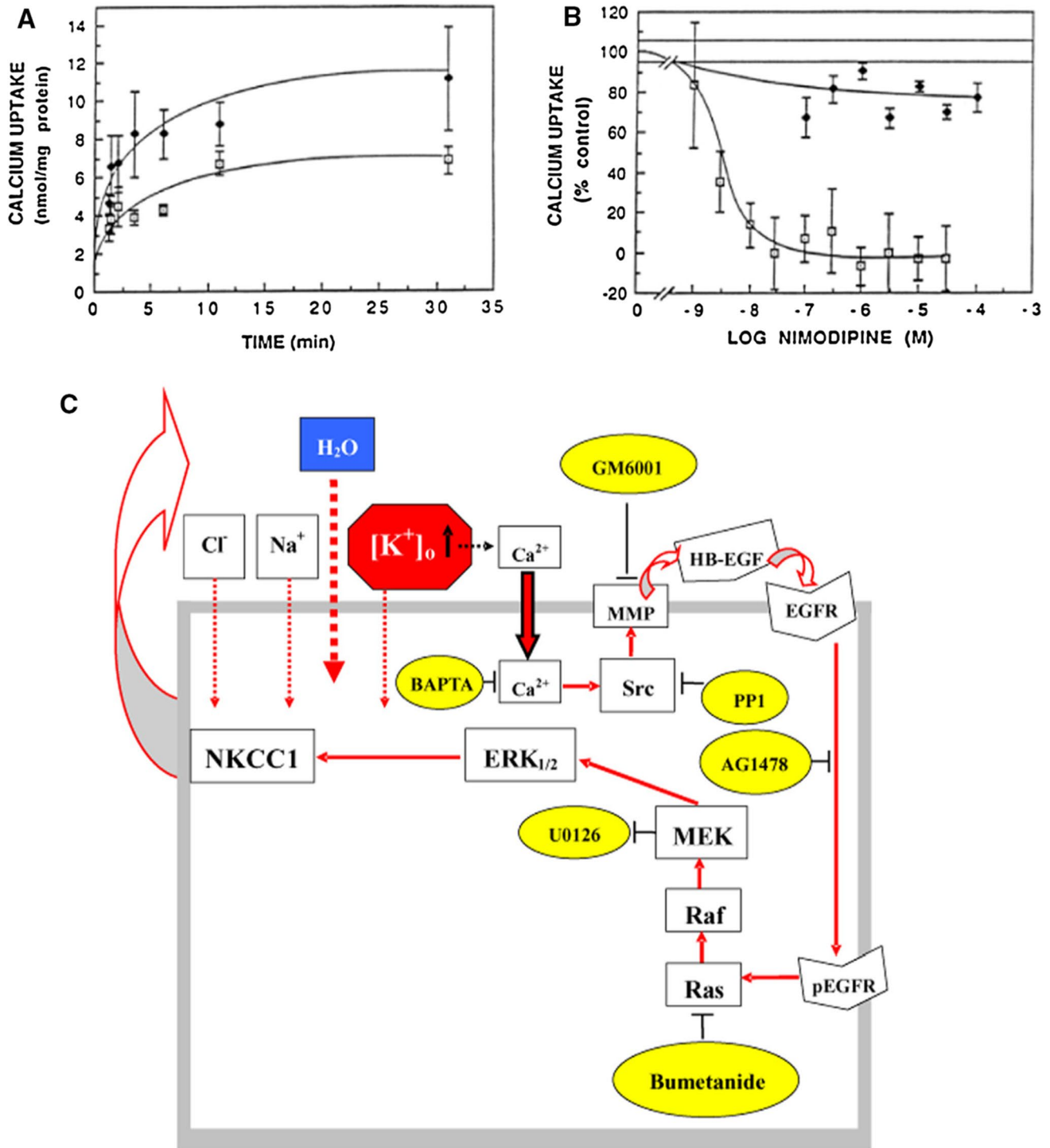
regulation [72]. The uptake velocity of GABA (measured at 50 mM) was also normal [4]. The contents of glutamate and glutamine showed also no significant difference (Table 2). There was no decrease in glutamine synthetase activity, consistent with a minimal effect on glutamate/glutamine labeling from acetate in Jimpy mice [49].

O₂ Uptake Stimulation by High [K⁺]_o Reflects L-Channel Mediated Ca²⁺ Uptake Stimulating Glycogenolysis, ATP Release and NKCC1

An increase in [K⁺]_o is a hallmark of neuronal excitation and it is evoked by several different mechanisms, including action potential generation (reviewed in [60]). The significance of most effects of highly elevated [K⁺]_o on astrocytes were unknown when the effect of elevated [K⁺]_o on oxidative metabolism in Jimpy mice were studied, but are a key to the interpretation of this phenomenon. Overwhelming evidence indicates that elevated [K⁺]_o at concentrations up to ~10 mM is initially accumulated by the K⁺-stimulated astrocytic Na⁺,K⁺-ATPase [64, 73–79] and subsequently released by Kir4.1 channels [76] over a wider area, securing little or no elevation of [K⁺]_o and allowing neuronal re-uptake [60]. Although this uptake can be fueled by oxidative metabolism of pyruvate [80], the rate of lactate production in brain slices [81] and cultured astrocytes [82] is high due to exit of lactate to the large surplus of medium and consequent

Table 2 Contents of glutamate and glutamine (µmol/100 mg protein) in cultured astrocytes from Jimpy mice and normal littermates

Amino acid	Jimpy	Normal littermates
Glutamate	2.04 ± 0.22 (n=6)	2.04 ± 0.19 (n=6)
Glutamine	8.02 ± 1.20 (n=4)	6.62 ± 0.71 (n=4)



abolition of the normal feed-back inhibition by pyruvate. A small increase in this glycolysis suffices to support Na^+ , K^+ -ATPase-activated K^+ uptake and there is no significant stimulation of rate of oxygen uptake in either brain slices or cultured astrocytes until $[\text{K}^+]_o$ becomes high enough to also stimulate the Na^+ , K^+ , 2Cl^- and water cotransporter NKCC1.

This cotransporter [61–64], which is metabolically driven by Na^+ , K^+ -ATPase-created ion gradients [67], is stimulated in cultured astrocytes by $[\text{K}^+]_o \geq 15 \text{ mM}$ [59, 60] and in brain slices by somewhat higher $[\text{K}^+]_o$ [55, 56, 83]. Expression of NKCC1 or its gene *Slc25A12* has been shown in cultured astrocytes [84] and oligodendrocytes [85–87] and in astrocytes in intact brain tissue by Kanaka et al. [88]

Fig. 7 a Uptake of ^{45}Ca into normal astrocytes in primary cultures as a function of time at 37°C at the normal extracellular K^+ concentration of 5.4 mM (*open squares*) or at 60 mM extracellular K^+ (*filled diamonds*) shown as means \pm SEM. ^{45}Ca (0.5 μCi) had been added to 1 ml serum-free medium 60 s before the increase in K^+ concentration. **b** Effect of addition 1 h before the measurements of nimodipine, a blocker of L-channels for Ca^{2+} , on unstimulated ^{45}Ca uptake (5.4 mM $[\text{K}^+]_o$, filled diamonds) and K^+ -stimulated ^{45}Ca uptake (60 mM K^+ , open squares) into cultured astrocytes shown as means \pm SEM. Note that only the latter is strongly and potently inhibited. **c** Diagram showing signaling pathways towards $\text{ERK}_{1/2}$ phosphorylation activated by elevation of the extracellular K^+ concentration and inhibition of this pathway by specific inhibitors (*ovals*). Elevation of the extracellular K^+ concentration above 10–15 mM depolarizes the cell membrane sufficiently to lead to depolarization-mediated Ca^{2+} entry through voltage-dependent L-channels. This effect increases with the magnitude of the increase in $[\text{K}^+]_o$ for which reason 60 mM $[\text{K}^+]_o$ was used in this study. The increase in $[\text{Ca}^{2+}]_i$ is necessary for $\text{ERK}_{1/2}$ phosphorylation, as indicated by its inhibition by BAPTA-AM. Although not shown in this Fig. the $[\text{Ca}^{2+}]_i$ increase is essential for glycogenolysis (see text) and for release of gliotransmitter ATP (Fig. 8). The increase in $[\text{Ca}^{2+}]_i$ also leads to a Src-dependent (and PP1-inhibited), release of the growth factor heparin-binding epidermal growth factor (HB-EGF). The released HB-EGF activates (phosphorylates) the EGF receptor (inhibited by AG1478), leading to activation of the MAP kinase cascade, Ras (inhibited by bumetanide), Raf and MEK (inhibited by U0126), with activation of MEK causing phosphorylation of extracellular regulated kinases 1 and 2 ($\text{ERK}_{1/2}$). $\text{ERK}_{1/2}$ phosphorylation is a major signaling event in CNS myelination [131], and it is also stimulated by astrocytic P2Y stimulation by ATP, which activates a pathway similar, but not identical to that shown in this Fig. [128]. $\text{ERK}_{1/2}$ activation also activates (phosphorylates) the cotransporter NKCC1 through pathways that were not studied and are only partly known. This leads to astrocytic influx of Na^+ and K^+ together with 2 Cl^- and water **a, b** from [101]; **c** from [60]

and MacVicar et al. [89]. mRNA and protein expression of NKCC1 has been determined in astrocytes of the dorsal root and trigeminal ganglia of the rat [90].

That the K^+ -induced stimulation of O_2 uptake in brain slices reflects stimulation of NKCC1 is shown by its inhibition by the non-specific NKCC1 inhibitor ethacrynic acid [91]. Stimulation by highly elevated $[\text{K}^+]_o$ develops between postnatal day 10 and 15 [92], i.e., at the same period when Jimpy mice begin to show their neurological symptoms. This phenomenon has also been observed in microdissected cat astrocytes [93] and in cultured rat and mouse astrocytes [54, 94]. The K^+ -mediated stimulation of O_2 uptake in brain slices is abolished in the absence of Ca^{2+} in the medium [55].

The reason why the K^+ -stimulated O_2 uptake in brain slices requires the presence of Ca^{2+} is that the stimulation of NKCC1 by elevated $[\text{K}^+]_o$ is due to depolarization-mediated activation of L-channel-mediated Ca^{2+} uptake. It is often believed that astrocytes have no functional L-channels, partly because Carmignoto et al. [95] reported the absence of voltage-dependent Ca^{2+} channels in astrocytes in brain slices. However these authors used 2 mM lactate

as the only metabolic substrate, and L-channel activity depends upon metabolism [96], in cultured astrocytes specifically degradation of glycogen [59], which is only slowly formed from lactate. Other authors have demonstrated Ca^{2+} channel activity in retinal glial cells [97], and Duffy and MacVicar [98] described an increase in $[\text{Ca}^{2+}]_i$ in hippocampal astrocytes in situ which was inhibited by the L-channel blocker verapamil. Finally, mRNA for the L-channels $\text{Ca}_v1.2$ and $\text{Ca}_v1.3$ are expressed both in cultured and freshly isolated mouse astrocytes [99, 100].

In cultured astrocytic depolarization by highly elevated $[\text{K}^+]_o$ leads to nimodipine-inhibited opening of L-channels for Ca^{2+} [101] (Fig. 7a, b). This activates a signaling pathway that includes transactivation [102–105] of the epidermal growth factor (EGF) receptor and eventually leads to phosphorylation and activation of NKCC1 and astrocytic swelling [106, 107] (Fig. 7c). It is unknown if the astrocytic ion and water changes or the accompanying alterations in the extracellular fluid affect oligodendrocytes or myelination. The growth factor released by K^+ -induced depolarization is heparin-binding epidermal growth factor (HB-EGF), as shown by ELISA. It is released from its membrane-bound precursor by the matrix metalloproteinase (MMP) ADAM17, and this process is prevented by the metalloproteinase inhibitor GM6001 and by siRNA against ADAM17 [103].

Figure 7c also shows that the pathway includes an increase in $[\text{Ca}^{2+}]_i$ and it therefore stimulates glycogenolysis, an effect that is inhibited by nifedipine at high $[\text{K}^+]_o$, but not at low $[\text{K}^+]_o$ [108, 109]. This is important because glycogenolysis is required for a multitude of astrocytic processes and signaling functions [110–113], including K^+ -stimulated release of transmitter ATP [114, 115]. Addition of 25 mM K^+ to brain slices also causes Ca^{2+} -dependent release of ATP, which mainly is astrocytic as shown by a drastic reduction of the release in the presence of fluoroacetate [116], a mitochondrial astrocyte-specific inhibitor. However, beyond (or perhaps because of) its dependence on glycolysis, K^+ -induced release of ATP in cultured astrocytes is also dependent on L-channel opening as seen by its abolishment by the L-channel inhibitor nifedipine (Fig. 8). Glycogenolytically generated lactate is released to the extracellular space where it stimulates neuronal signaling [110, 117–119].

It has been disputed whether the increases in $[\text{K}^+]_o$ during normal neuronal stimulation in brain slices are high enough to activate NKCC1 [77]. However, the increase in $[\text{K}^+]_o$ in the narrow space between astrocytes or either neurons or oligodendrocytes might be higher than those generally measured. Moreover, transmitter-mediated inhibition of gap junction coupling may facilitate K^+ -mediated L-channel opening [60]. This concept is supported by an activity-dependent decrease in extracellular space in rat

optic nerve, which paralleled the increase in $[K^+]_o$, and ontogenetically developed *pari passu* with the appearance of glia cells [120]. A comparison between the ontogenetic development of the extracellular shrinkage and the development of astrocytes and oligodendrocytes suggested that swelling of both of these two glia cell types (both of which express NKCC1) may be responsible for the reduced extracellular space.

It is in further support of a physiological role of depolarization-mediated opening of L-channels for Ca^{2+} that 2 weeks of fluoxetine treatment increases $Ca_v1.2$ expression not only in cultured astrocytes but also in astrocytes in brain [100]. This would probably not have been the case if the channel was not operating. $Ca_v1.2$ mRNA is also up-regulated in hyperammonemic mice [121], consistent with the ability of NH_4^+ to potently stimulate NKCC1 via opening of L-channels.

Release of Transmitter ATP from Axons and Astrocytes Promotes Oligodendrocyte Development and myelination

The only demonstrated metabolic defect in Jimmy astrocytes was the absence of stimulation of oxygen uptake

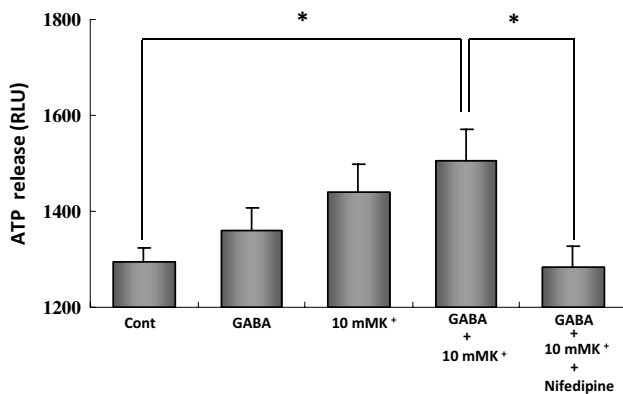


Fig. 8 Release of gliotransmitter ATP was determined as relative light units (RLU) measured by a luciferin/luciferase technique in cultured astrocytes during incubation for 60 min in glucose-containing (7.5 mM) Dulbecco's medium supplemented with the ecto-ATPase inhibitor ARL67156 to prevent degradation of released ATP. Addition of 10 mM K^+ alone (to a final level of 17.5 mM) or the combined addition of K^+ and GABA caused a significant increase in ATP release, although to a smaller degree than higher, more depolarizing K^+ concentrations (shown in [114]). This increase was abolished in the additional presence of the L-channel inhibitor nifedipine. The glycogenolysis inhibitor 1,4-dideoxy-1,4-imino-D-arabinitol (DAB) also inhibited K^+ -induced ATP release (shown in [114]). RLU values are only shown above 1200 RLU because this level was reached also under control conditions. Results are the averages of RLU values from three to five individual cultures. S.E.M. values are indicated by vertical bars. *Statistically significant ($P < 0.05$) difference from all other groups, but not from each other. From [114]

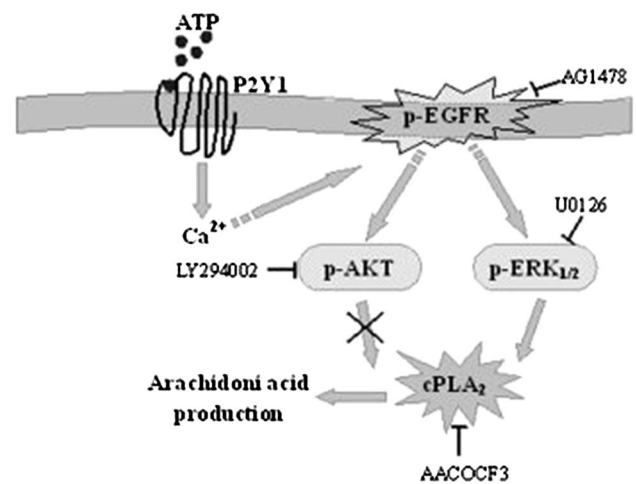


Fig. 9 Proposed signal transduction pathway activated by extracellular ATP in spinal cord astrocytes. ATP binds to the P2Y1 purine receptor, which increases $[Ca^{2+}]_i$ and causes phosphorylation of the EGF receptor by a process reminiscent of that shown in Fig. 7c. Activation of the EGF receptor stimulates phosphorylation of ERK_{1/2} and Akt and stimulation of cPLA2, leading to release of arachidonic acid, all events that seem important for oligodendrocytic proliferation and survival and myelination. Selective inhibitors used to establish the pathway are shown by —|. From [128]

by highly elevated $[K^+]_o$, which as discussed above is an indication of lack of L-channel-mediated Ca^{2+} uptake. In turn this will abolish the normal stimulation of glycogenolysis and of ATP release. In the isolated optic nerve action potentials trigger release of ATP, which acts on astrocytic purinergic receptors to further enhance ATP release and to initiate intercellular Ca^{2+} waves to neighboring glia, including oligodendrocytes and NG2 cells [122, 123]. Combined axonal/astrocytic release of ATP activates oligodendrocytic purinergic receptors and regulates myelination [124–127]. In both astrocytes and oligodendrocytes moderate quantities of ATP act predominantly via the G protein coupled P2Y purinergic receptors. In cultured spinal astrocytes this causes a rise in $[Ca^{2+}]_i$ (Fig. 9) through IP_3 -dependent release of Ca^{2+} from intracellular stores with further growth factor-mediated down-stream activation of extracellular-regulated kinases 1 and 2 (ERK_{1/2}) [128]. This process reminds of that shown in Fig. 7c, a rather similar pathway also stimulates DNA synthesis in retinal Müller cells although the metalloproteinase is MMP 9 [129]. Growth factor formation may also be essential for oligodendrogenesis and myelination. Thus, in preterm infants intraventricular hemorrhage causes a decline in epidermal growth factor (EGF) and myelination, and in a rabbit model of this condition recombinant human EGF enhances oligodendrocytic proliferation and maturation, myelination and neurological recovery [130]. This may reflect that ERK_{1/2}

phosphorylation is a major signaling event in CNS myelination [131]. It is also consistent with the conclusion that P2Y stimulation generally has beneficial effects on oligodendrocytic maturation and survival and on myelination [127]. Many of the ionotropic P2X receptors are activated at low ATP concentrations, but P2X7 receptors are stimulated at high concentrations of ATP, resulting in sustained influx of Ca^{2+} which has adverse effects [127].

Xia and Zhu [128] also showed that ATP stimulates phospholipase A_2 (Fig. 9), which releases arachidonic acid, a component of myelin [132] and that it activates Akt, which in turn decreases phosphorylation of glycogen synthase kinase β (GSK β) [133]. Akt has many known down-stream effects in oligodendrocytes [76] and a decrease in GSK β would also be beneficial, since Azim and Butt [134], showed that knock-out of cyclin-dependent kinase 5 (Cdk5) impairs myelin repair by reducing signaling through the Akt pathway which will enhance GSK3 β signaling [135]. Azim and Butt [134] investigated effects of GSK3 β inhibition on oligodendrocyte development from their precursors in vivo by injection into the lateral ventricle of post-natal mice and in organotypic cultures of intact optic nerve. Their results showed that each of the GSK3 β inhibitors ARA-014418, indirubin, L803-mt and Li^+ increased oligodendrocyte precursor cells and oligodendrocytes and promoted myelination. Since intact tissue was used it can not be excluded that the target of the inhibitors included astrocytes, where GSK β inhibition would stimulate glycogen synthesis [136, 137], facilitating ATP release. Absence of β_2 -adrenergic receptors in white matter astrocytes in multiple sclerosis patients [138] might also affect myelination by inadequate glycogenolysis (although most evidence suggests that glycogenolysis in brain is mediated by stimulation of β_1 -adrenergic receptors [108, 109]). Reduced glycogenolysis would diminish glycogen-derived increases in extracellular lactate and the associated signaling.

Another major reason for the beneficial effect of ATP on oligodendrocytes and myelination is probably that it greatly increases the expression of mRNA for leukemia inhibitory factor (LIF) in cultured astrocytes [139, 140]. Secretion of LIF from astrocytes is also stimulated in response to transmitter ATP released from axons during firing of action potentials in an intricate communication between axons and myelinating glial cells [139, 141]. Oligodendrocyte precursor co-culturing with astrocytes similarly enhances expression of LIF [142], and LIF $^{-/-}$ mice have a pronounced defect in oligodendrocyte maturation and myelination, with strongly reduced PLP and MBP contents in optic nerve at postnatal day 10 [143]. One mechanism for the favourable effect of LIF is that it induces the neuropeptide galanin which is a survival factor in oligodendrocytes [144].

Knockout of Oligodendrocytic Connexins Causes Jimpy-like Symptoms

Treatment of mouse embryonic stem with LIF together with bone-morphogenic protein-2 (BMP-2) causes a large increase in Cx43 expression in cardiomyocytes [145]. It is unknown if this also applies to astrocytes and/or oligodendrocytes and to different connexins. However, a symptomatology strikingly similar to that in Jimpy mice, except that the mice may live slightly longer, has been found in mice with double knockout of the oligodendrocytic connexins Cx32 and Cx47. The knockout causes early CNS demyelination and oligodendrocytic death [146, 147]. Vacuoles develop in these mice, mainly in the periaxonal space. They are exacerbated by increased activity, but greatly reduced by tetrodotoxin (TTX) and separate the axons from their myelin sheaths [146, 147]. The dysmyelination and formation of vacuoles is accompanied by the development of a progressive tremor during the third postnatal week followed by tonic seizures, which increase until the animals die, typically during the sixth postnatal week [148, 149]. Menichella et al. suggested that the vacuoles (and thus perhaps also the seizures) reflected pathological accumulation of ions and H_2O which the mutant mice are unable to redistribute [148]. They also pointed out that vacuolated myelin similarly has been reported in mice lacking the K^+ channel Kir4.1 [150], which is expressed in both oligodendrocytes and astrocytes. They therefore performed an ultrastructural examination in P9 or P10 of Kir4.1 $^{-/-}$ mice (which die soon thereafter and thus do not live long enough to show neurological dysfunction), which showed abundant vacuoles in the spinal cord white matter. However, in contrast to the periaxonal localization of the vacuoles in the Cx32/47 double knockouts these vacuoles were generally located on the outside of the myelin sheaths, showing that ions and water had been able to penetrate the myelin sheath but not get any further. Based on these results Menichella et al. [148] proposed a pathway that included oligodendrocyte-astrocyte gap junctions which would be interrupted in the mutants and concluded that oligodendrocytes and their connexins have a critical role in K^+ homeostasis in myelinated neurons. To assess this conclusion astrocytic and oligodendrocytic connexins will be described in some detail and they are illustrated in Fig. 10.

It has long been known that oligodendrocytes express gap junctions which connect them with astrocytes [151–153]. These early authors did not show gap junctions between oligodendrocytes but intensive inter-oligodendrocytic coupling has later been found to occur via Cx47:Cx47 and Cx32:Cx32 homotypic gap junctions [154, 155]. At least Cx32 also forms hemichannels [156] which may allow exit of cytosolic molecules, possibly including K^+ . In cerebellum of the double Cx47 and Cx32 knockouts, Wasseff

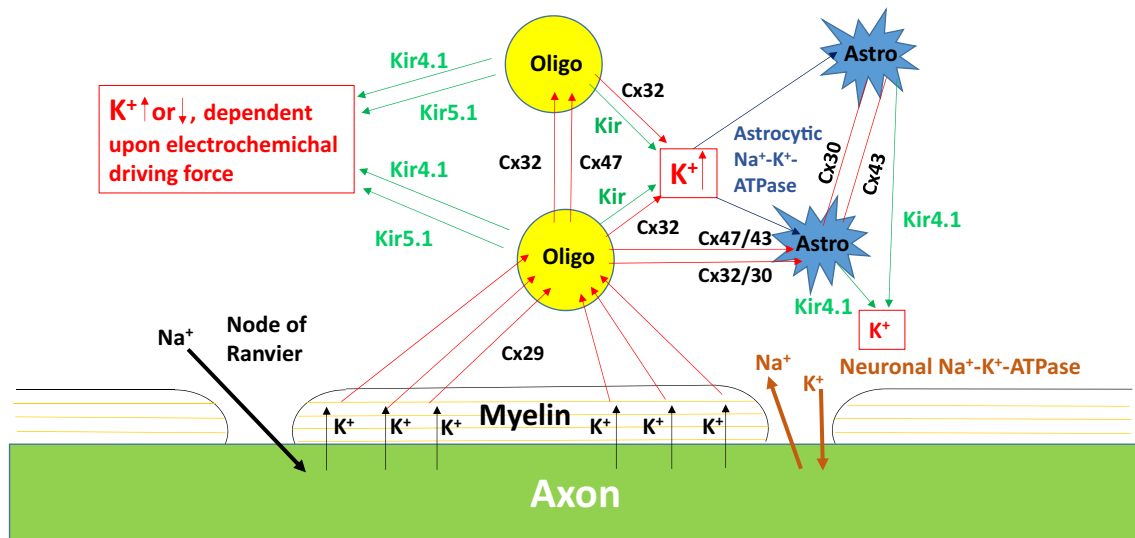


Fig. 10 Schematic illustration of proposed involvement of K^+ channels, connexins and Na^+ , K^+ -ATPase in myelin, oligodendrocytes and astrocytes in K^+ homeostasis in normal myelinated neurons, with the exception that oligodendrocyte-astrocyte connection might be slightly more flexible than indicated, and that K2P channels might also play a role. The axon is depolarised by Na^+ entry at one node of Ranvier (black arrow). Gradual repolarisation occurs by K^+ release via K_v channels under the compacted myelin sheath of the internode (black arrows), a localisation which in the CNS may be especially pronounced in the juxtaparanodal region. Released K^+ is carried by Cx29 (red arrows) into oligodendrocytes and possibly redistributed into several oligodendrocytes by Cx32 and 47 (red arrows). K^+ is transported in or out of oligodendrocytes by Kir channels (green arrows) and it is also released by Cx32 hemichannels, leading to an increase in $[K^+]_o$ with subsequent uptake into astrocytes by the astrocytic Na^+ , K^+ -ATPase (blue arrows), which is stimulated by elevated $[K^+]_o$. Redistribution between additional astrocytes occurs via Cx30 and 43. Subsequently accumulated K^+ is released from astrocytes via Kir4.1 and due to the redistribution over a larger area $[K^+]_o$ may not

and Scherer [157] found increased expression of 348 genes together with decreased expression of 23 genes, many of them oligodendrocytic and two involved in synthesis of myelin lipids. On the other hand co-culture of oligodendrocyte precursor cells with astrocytes induces upregulation or induction of ~50 genes related to myelination, including enhancement of the function of Cx 29 and Cx47 [142].

The main connexin in astrocytes is Cx43 but they also express Cx30 [158–160] together with several other connexins [156], and coupling between astrocytes is mediated by homotypic channels (e.g., Cx43/Cx43 and Cx30/Cx30) [161–163]. Very efficient gap junctions between astrocytes and oligodendrocytes are formed by Cx47/Cx43, whereas transport through Cx32/Cx30 gap junctions is somewhat more restricted, and other Cx combinations do not form gap junctions between the two glia cell types [164, 165]. Somewhat larger variability was found in HeLa cells expressing glial connexins, where Cx47 forms heterotypic channels with astrocyte Cx43 or Cx30, whereas Cx32 can

become significantly reduced, furthering re-uptake by the neuronal (axonal) Na^+ , K^+ -ATPase (brown arrows), which is fully activated at normal $[K^+]_o$. A partly similar pathway has been suggested by Menichella et al. [148], but they suggested Cx-mediated K^+ transfer from oligodendrocytes to astrocytes. For reasons indicated in the text we do not believe such a net transport occurs. The oligodendrocytic Cx-mediated K^+ release via Kir channels or Cx hemichannels, followed by astrocytic Na^+ , K^+ -ATPase-mediated accumulation explains the lack of K^+ efflux at the node of Ranvier. It is also consistent with findings by Ransom et al. [73] that K^+ released from the optic nerve is initially accumulated by astrocytes and secondarily by the axon, and the subsequent release via Kir4.1 channels is consistent with results by Bay and Butt [76]. In Cx 32/47 knockouts oligodendrocytic redistribution and hemichannel-mediated K^+ release is impaired, leading to loosening of myelin and K^+ diffusion along the axon and probably also prevention of saltatory impulse propagation. Approximately similar consequences can be expected in Jimpy mice due to failing myelination (Fig. 2) or lack of a compacted myelin sheath

functionally interact with astrocyte Cx30 or Cx26 but not Cx43 [166].

If the reason for the severe seizure activity of mice with double knockout of the oligodendrocytic Cx32 and Cx47 were failing communication between oligodendrocytes and astrocytes via these connexins, double knockout of the astrocytic Cx43 and Cx30 could be expected to have a similar effect, unless their function could be taken over by Cx26. However, in spite of vacuoles in the oligodendrocytes, which were interpreted as possibly due to decreased K^+ buffering, such mice did not convulse or die early, although they were impaired in balance and spatial memory tasks [167]. This rather mild symptomatology makes it unlikely that the reason for the severe Jimpy-like symptoms in the Cx32/47 knockouts should be an interruption of K^+ trafficking from oligodendrocytes to astrocytes via gap junctions as suggested in [148]. Moreover, K^+ uptake, measured by uptake of ^{42}K is much slower in cultured oligodendrocytes than in cultured astrocytes, and so is the

final level of ^{42}K reached, an indication that the intracellular K^+ concentration also may be much lower than that in astrocytes [168]. If this also is the case in vivo there can be no net transport of K^+ from oligodendrocytes to astrocytes via gap junctions. Surprisingly there was also very limited gray matter pathology as a result of the knock-out of the astrocytic connexins, possibly due to the expression of additional connexins in astrocytes, which may have been upregulated.

Cx29 is expressed in oligodendrocytes [149, 164] as well as in myelin throughout the brain and spinal cord [169] where antibody labeling shows its location at internodal and juxtaparanodal regions of small myelin sheaths [170]. Immunofluorescence for Cx29 shows minute puncta within oligodendrocytes and around their periphery. The labeling for Cx29 in oligodendrocyte somata is intense at young ages but it is dramatically shifted to be localized primarily in myelinated fibers in the mature CNS. Cx32 labeling is also localized to oligodendrocyte somata and myelin, but Cx29 and Cx32 are minimally co-localized on oligodendrocyte somata although partly co-localized along myelinated fibers [171]. Ahn et al. also reported that Cx29 does not form gap junctions with other connexins in oligodendrocytes [172] in spite of the oligodendrocytic expression of Cx32 and CX47. In a peripheral nerve Rash et al. [173] found that Cx29 in the juxtaparanodal myelin collars and along the inner mesaxon is precisely aligned with and ultrastructurally coupled in a 1:1 ratio to internodal K_v channels. From these findings [170, 171, 173] it can be concluded that molecules carried from myelin may end up in the oligodendrocytic cytoplasm. This may include K^+ released via internodal K_v channels because connexins mediate K^+ flux [174].

Is K^+ Homeostasis Compromised in Jimpy and Cx32/47 Knockouts?

ATP signalling regulates K^+ channels (Arthur Butt, Portsmouth University website), which interact with connexins and with the Na^+, K^+ -ATPase in K^+ transport between glia cells and extracellular fluid [60, 76, 77]. Wallraff et al. [175] examined changes in K^+ homeostasis in hippocampal slices of mouse astrocytes, which were deficient in astrocytic gap junction coupling due to a double knockout of connexins 43 and 30. The knockout increased $[\text{K}^+]_o$ during intense neuronal firing (in some case above the normal ceiling of 12 mM), reduced the threshold for generation of epileptiform events, and caused decreased K^+ clearance, especially via the fast component of the K^+ uptake. This component has been shown [73] to reflect active uptake into astrocytes, but since the accumulated K^+ normally is re-released by Kir4.1 channels [76], possibly after

Cx-mediated redistribution [60] it may well be affected by Cx knockout. That even small elevations of $[\text{K}^+]_o$ in hippocampus have epileptogenic effect has repeatedly been observed [176–179]. Potential effects exerted on myelinated nerves or glia cells were not discussed, but Hubbard and Binder [180] have reviewed astrocytic roles in epilepsy, and we have recently discussed roles of astrocytes in the adult brain in K^+ homeostasis [60].

Here we will consider possible functions of connexins and K^+ channels in myelinated axons and oligodendrocytes and their interaction with Na^+, K^+ -ATPases for Na^+/K^+ homeostasis, which accounts for a large fraction of brain energy metabolism. Since temporally Jimpy pathology is restricted to the last 3 weeks of the first postnatal month it is important that brain oxygen consumption in all species increases with development [181] and in the rat (which developmentally is rather similar to the mouse) increases 2–3 times between postnatal days 10 and 20. This explains why myelination, which enables saltatory impulse propagation that energetically is much less costly than the contiguous impulse propagation in non-myelinated axons develops postnatally. It is probably also the reason why Jimpy animals show no symptoms during the first 1–2 weeks of life, but develop seizures at a time when myelination is abnormal due to failing myelination and/or compaction of their myelin sheath [30] (Fig. 2). As a result, contiguous impulse propagation probably continues with large passive of Na^+ entry and K^+ exit and subsequent costly energetic reversal of the passive ion fluxes by the Na^+, K^+ -ATPase which develops with a similar pattern as oxygen consumption [181]. Since astrocytes are relatively scarce in white matter [182], and the initial removal of increased extracellular K^+ is astrocytic [60] due to K^+ -induced stimulation of the astrocytic but not of the neuronal Na^+, K^+ -ATPase, this may possibly lead to increased $[\text{K}^+]_o$. In turn, the increased $[\text{K}^+]_o$ may evoke seizures. The probable absence of NKCC1 which contributes to K^+ accumulation at high $[\text{K}^+]_o$ may further undermine K^+ uptake. Alternatively, in partly myelinated axons loss of the tight connection between the axon and the compacted myelin sheath [23, 24] may have similar effect as will be described below for Cx32 and 47 double knockout.

The symptomatology in mice with Cx32 and 47 double knockout of the oligodendrocytic Cx32 and Cx47 cannot be explained in a similar manner, since failing myelination has not been reported in these animals, although myelin sheaths are thinner in mice with double knockout of Cx47 and the astrocytic Cx30 [183]. Since we found it unlikely that the severe Jimpy-like symptoms in the Cx32/47 knockouts should be due to interrupted K^+ transport from oligodendrocytes to astrocytes via gap junctions, different possibilities for impaired K^+ homeostasis in myelinated axons will be considered. Profound

differences exist in characteristics of the myelin producing cells in the central (oligodendrocytes) and peripheral (Schwann cells) nervous systems [184] as well as in myelin structure at the two different locations [26]. Nevertheless, some features could be common, perhaps including the important association between K_v channels and Cx29 recently described by Rash et al. [173], but in general we will focus on K^+ handling in the CNS.

The myelin wrapping of the axon is interrupted by nodes of Ranvier, which usually extend for $100\ \mu\text{m}\sim 1\ \mu\text{m}$ along the fiber axis and are separated by internodes of $\sim 1\ \text{mm}$ length. The axon membrane (the axolemma) is highly differentiated at the nodes of Ranvier, exhibiting different properties in that region compared to other sites along the fiber, including a high density of voltage-dependent Na^+ channels (Na_v), but no voltage-dependent K^+ channels (K_v) [185]. This led to the concept that repolarization exclusively occurs by an outward leakage of K^+ [185]. A two-pore domain K^+ channel (K2P) has been shown to enable action potential generation in the absence of K_v [186], but this was demonstrated in HEK cells, and this channel has never been shown immunohistochemically in myelinated neurons. Moreover K_v channels are normally present under the myelin sheath in the paranodal and internodal axon membrane of rabbit nerve fibers, and voltage clamp studies have demonstrated the appearance of K_v conductance in mammalian nerve fibers after sudden disruption of the myelin sheath [187]. These K_v channels might well be of functional importance, on account of the previously described transport of K^+ into oligodendrocytes by Cx29 located internodally and contacting K_v channels in a 1:1 ratio [173] without being in direct contact with the oligodendrocytic Cx32 [172]. In the myelinated fibers of the CNS the K_v channels may be clustered at the juxtaparanodal region reached before the next node of Ranvier [26, 188–190], rather than located under the entire internode as shown in Fig. 10. However, immunocytochemistry might more easily reveal juxtaparanodal than internodal channels, and uniform calcium influx along the entire stimulated axon of a fully myelinated mouse optic nerve [191] suggests K_v channel localization under internodal myelin also in the CNS. This is especially the case since cadmium inhibition of the calcium transients suggests that calcium channels are involved, although nifedipine had only a minor inhibitory effect [191], possibly due to limited access. Anyhow, internodal versus juxtaparanodal localization would probably not be of major functional importance, as long as the channels are located before the next node of Ranvier. Myelin compaction and the formation of tight axoglial junction is required to create and maintain the location of the K_v channels under the myelin sheath [188]. In Jimpy mice nodal expression of Na^+ channels is normal,

but the paranodes are not intact as indicated by elimination of normal components [192], which suggests abnormal K_v function.

The normal localization of K^+ channels and their association with connexins is consistent with the periaxonal vacuoles found in the Cx32 and 47 double knockouts [146, 147]. Subsequently K^+ may be transported out of oligodendrocytes by homomeric Kir4.1 or heteromeric Kir4.1/Kir5.1 channels localized at their membrane [193]. Interference with oligodendrocytic efflux would in the long run increase K^+ in oligodendrocytes and thereby prevent K^+ transport from the outer surface of myelin into oligodendrocytes, which would explain why *Kir4.1*^{-/-} mice show vacuoles at the myelin surface [148]. The suggested pathway would also be consistent with the observations that optic nerve activity increases $[\text{K}^+]_o$ and that this increase stimulates Na^+, K^+ -ATPase-mediated K^+ uptake, first in astrocytes by the potent K^+ -stimulated Na^+, K^+ -ATPase in astrocytes [73, 78, 194] and subsequently in neurons after Kir4.1-mediated release of K^+ accumulated in astrocytes [60]. Repolarising, gradual K^+ exit via K_v channels dispersed along the internodal axon may be important for saltatory impulse propagation, since it is likely to make the axon independent of renewed excitation until full repolarisation has been achieved immediately before the next node of Ranvier. Such a function of myelin-covered K_v channels would not exclude additional operation of leakage channels at the nodes, especially if full repolarisation has not been achieved. If normal K^+ homeostasis fails, due to inefficient transport across the myelin sheath by Cx29, exit from oligodendrocytes via Kir channels or dysmyelination, it is likely that K^+ would accumulate periaxonally and reach the node of Ranvier by diffusion between the axon and a loosened myelin sheath. This may have consequences for impulse conduction, energy metabolism and seizure susceptibility. The risk for development of seizures might be especially pronounced if myelinated neurons within gray matter are affected, and an enhanced metabolic requirement after abrogation of saltatory conduction might expose the tissue to glutamatergic excitation.

Concluding Remarks

Since the stimulation of O_2 uptake in Jimpy mice by highly elevated $[\text{K}^+]_o$ represents L-channel opening, the conclusion that depolarisation by high $[\text{K}^+]_o$ is unable to activate L-channel-mediated Ca^{2+} uptake in Jimpy astrocytes seems valid. Nevertheless this should be confirmed experimentally, probably most easily by determining effects of K^+ addition on glycogenolysis in brain tissue, remembering the slow regeneration of glycogen in slices after their preparation [195, 196]. Since most of the K^+ -induced release of

transmitter ATP comes from astrocytes [116], it can also be expected that the effect of elevated $[K^+]_o$ on ATP release will be greatly reduced. Sub-myelin K_v channels are very rare in Jimpy but occasionally found in one of the few myelinated axons [188].

Jimpy is generally not regarded as a model of human demyelinating disease. However, the childhood leukodystrophies have diverse molecular and genetic etiologies in spite of their common clinical features [197], and some of them are associated with mutations in connexin 47 [198–200]. That neonatal motor disturbances can result from deficient L-channel activity is shown by a higher frequency of motor disturbances in women treated with nifedipine to delay pre-term birth than in those treated with placebo [201]. This effect could equally well be exerted on astrocytes or their precursors as on neurons or oligodendrocytes or their precursors. Jimpy is the convulsant mutant where most knowledge of astrocytic abnormalities is available. Further studies of this mutant may therefore be clinically relevant for elucidation of physiological and pathophysiological roles of interactions between oligodendrocytes and astrocytes and their importance in the pathogenesis of leukodystrophies.

The very close similarity between neurological dysfunction in Jimpy and in mice with double knockout of the oligodendrocytic connexins Cx32 and Cx47 provides information about the possible pathophysiology in both conditions. Literature data about localization, especially of Cx29, and its possible interaction with K_v channels also in oligodendrocytes had led to the model of potential pathways involved in K^+ homeostasis shown in Fig. 10, but this pathway obviously needs experimental verification.

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