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Intracellular ion homeostasis

Impairment in hepatic encephalopathy

Hepatic encephalopathy

The term hepatic encephalopathy (HE) refers to a number of potentially reversible neurological deficits resulting from liver dysfunction [11]. Chronic HE, as seen in alcohol-induced liver cirrhosis, is characterised by personality changes, altered mood and diminished intellectual ability.

Acute HE occurs following acute liver failure, resulting from for example viral hepatitis and paracetamol intoxication, is associated with neurological deficits ranging from impairment of fine motor skill to muscle spasticity [uncoordinated movements (ataxia)], and finally impaired consciousness and coma. In most patients with acute liver failure, cerebral oedema ensues and is the predominate cause of death (80–90% mortality rate). The only effective treatment presently available for the cerebral oedema in ALF is an emergency liver transplantation.

Ammonium's central role

Ammonium (NH_4^+ and ammonia (NH_3) are considered the primary toxins responsible for the functional deficits observed in HE, the treatment of which is based primarily on reducing blood and brain NH_4^+/NH_3 concentrations. The arterial concentration of NH_4^+/NH_3 in HE patients and HE animal models is increased, and predicts the severity of the symptoms [1]. In the portocaval anastomosis animal model of chronic HE, a surgically placed shunt allows blood from the intestine to bypass the liver (unfiltered) and enter the main circulatory system, increasing arterial NH_4^+/NH_3 concentrations. Under physiological conditions, NH_4^+/NH_3 concentrations in arterial blood are 50–100 µmol/l; this level doubles in chronic HE, while in the central nervous system it rises three- to four-fold. In contrast, at the coma stage of acute HE the NH_4^+/NH_3 concentrations in the CNS of animal models reach 1–5 mM [1].

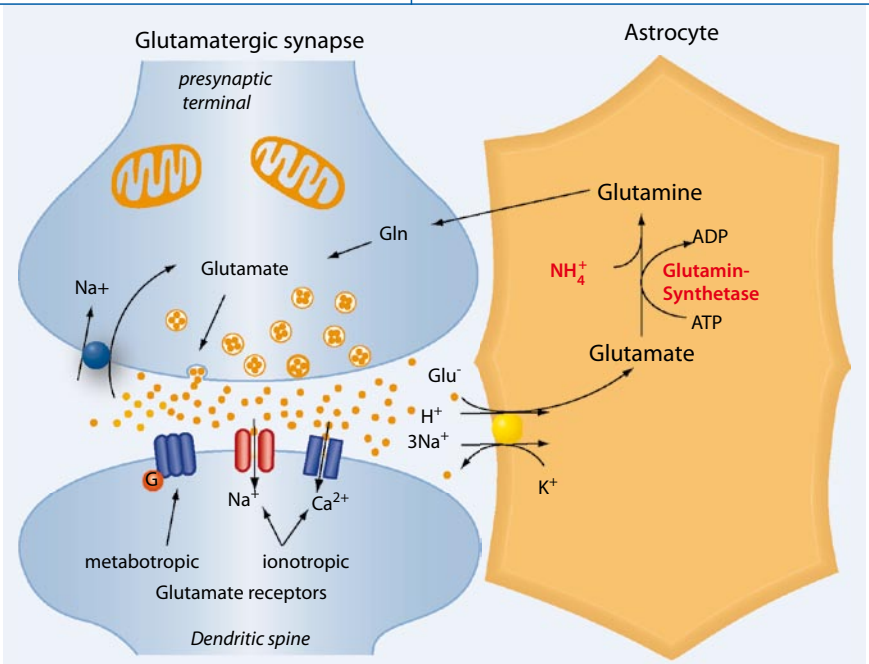
Astrocytes and HE

Recent evidence suggests that astrocytes are an important cellular site of action for NH_4^+/NH_3 in the pathology of HE (see [1, 3]). In particular, astrocytic swelling observed in animal models following exposure to NH_4^+/NH_3 likely contributes to the brain oedema, a frequent cause of mortality in acute HE. In addition, neuronal loss is rarely observed in the mature brain following NH_4^+/NH_3 exposure [1], and the neuropsychiatric symptoms characteristic of acute or chronic HE are reversible following recovery of hepatic function [11].

The greater sensitivity of astrocytes to NH_4^+/NH_3 compared with neurones likely reflects the mechanism of NH_4^+ cellular influx (see below) and/or astrocyte specific pathway(s) particularly sensitive to NH_4^+/NH_3. In regard to the latter, astrocytes are the cellular site for NH_4^+ detoxification in the CNS. Ammonium is detoxified in the CNS by the enzyme glutamine synthetase, which is located almost exclusively in astrocytes. In this process, the conversion of glutamate to glutamine incorporates ammonium, glutamine is converted back into glutamate after neuronal uptake (glutamate-glutamine shuttle; see Box. 1). Furthermore, increases in cellular glutamine concentrations are a consistent hallmark of HE.

Ammonium additionally influences astrocyte volume regulation. The intracellular accumulation of glutamine contributes to astrocytic swelling, which is counteracted by release of osmolytes such as myoinositol and taurine. Astrocytic swelling leads to production of reactive oxygen and nitrogen compounds, which in turn exacerbate the swelling [4]. In the case of acute HE, astrocyte swelling affects both the cell bodies and their branches and may be so extensive that the fatal cerebral oedema mentioned above ensues [1]. Furthermore, bolus injection of NH_4^+/NH_3, a model of acute HE, causes cerebral swelling in animal models primarily as a result of astrocyte swelling.

In the case of chronic HE, a milder form of astrocyte swelling is observed [3]. Chronic HE is characterized by Alzheimer type II astrocytes, the characteristic features of which include swollen cell nucleus, a prominent nucleolus and margination of the chromatin pattern [1]. Indeed, Alzheimer type II astrocytes are induced by chronic NH_4^+/NH_3 exposure both in HE animal models and cell cultures.



Umkehrpotential für den Glutamattransport (nach Zerangue & Kavanaugh, Nature 383, 1996); *n = Anzahl pro Zyklus transportierter Ionen*

$$E_{rev} = (RT/F\,(n_{Na} + n_H - n_K - n_{Glu}))\ \ln\left(([Na_o]/[Na_i])^{nNa} \times ([Glu_o]/[Glu_i])^{nGlu}\,([H_o]/[H_i])^{nH}\,([K_i]/[K_o])^{nK}\right)$$

Fig. 1 ▲ Reversal potential for glutamate transport. *n* The number of transported ions per cycle. (Modified according to [16])

HE and glutamate transmission

Another hallmark of HE is an increased extracellular glutamate concentration and altered glutamatergic neurotransmission (see [7]). Following bolus injection of NH_4^+/NH_3 into the bloodstream to trigger acute HE, the extracellular glutamate concentration increases within 20 min, attaining a peak 60 min post injection. Furthermore, neuronal NMDA (N-methyl-D-aspartate) receptors were activated within a similar time period. Increases in extracellular glutamate concentration may result from increased glutamate release or reduced glutamate uptake, and experimental evidence exists supporting both mechanisms [7]. Interestingly, a number of studies reported that NH_4^+/NH_3 evokes glutamate release from astrocytes in a Ca^{2+} dependent manner ([8, 2, 7]; see below). In addition, reductions in glutamate uptake were observed in animal models and in cultured astrocytes following chronic NH_4^+/NH_3 exposure and were attributed to reductions in the expression of glial glutamate transporters (see [7]; Box. 1).

Box 1: The glutamate-glutamine cycle, glial glutamate uptake and HE

Glutamate released at presynaptic terminals acts on metabotropic and ionotropic receptors, eliciting a response in the postsynaptic cell (◘ **Fig. 1**). Concurrent to the action of glutamate on glutamatergic receptors is the removal of glutamate by high-affinity transporters found primarily in astrocytes.

Glial glutamate uptake is predominantly mediated by glutamate transporters EAAT1 and 2 (excitatory amino acid transporters), which in the forward-mode require the co-transport of one proton and three sodium ions into and counter-transport of one potassium ion out of the cell. Furthermore, the electrogenic transport of glutamate, or more directly the reversal potential (E_{rev}) of glutamate uptake as described by Eqn. 1, is primarily dependent upon the inward sodium electrochemical gradient (see [16]). Thus, elevations of intracellular sodium concentration in glial cells shift the reversal potential for transport in the negative direction, thereby reducing the cells ability to take up glutamate.

In astrocytes, glutamate in combination with NH_4^+ is converted by the enzyme glutamine synthetase to glutamine, which is in turn exported to neurones where glutamine is involved in neuronal glutamate synthesis. The conversion of glutamate to glutamine in astrocytes is the only pathway for the removal of ammonium in the CNS, and increases in NH_4^+/NH_3 concentrations are observed in the CNS of HE patients. A hallmark of HE is an increased intracellular glutamine: glutamate ratio. In addition, increases in extracellular glutamate concentrations are consistently observed in HE, and result from increased glutamate release and/or reduced glutamate uptake. The increased glutamine concentration contributes to astrocyte swelling, an additional hallmark of HE.

Ion gradients and ion transport

A possible cause of HE-induced changes in glial cell function that has to date received little attention is changes in ion gradients and intracellular ion homeostasis. The production and maintenance of ion gradients across the plasma membrane is a fundamental requirement for the generation of the cellular membrane potential, for electric signalling and for transport processes coupled to ion gradients. In this regard, Na^+/K^+-ATPase activity is of particular importance. Na^+/K^+-ATPase activity exchanges intracellular sodium ions for extracellular potassium ions with a ratio of 3:2, respectively, and consumes ATP in the process. A multitude of secondary and tertiary active transporters utilise the inward sodium gradients produced by Na^+/K^+-ATPase activity to establish and further the gradients for potassium, magnesium, proton, bicarbonate, calcium or chloride ions.

The activity of these transporters results in specific differences between the extra- and intracellular concentrations of the transported ions. While the extracel-

lular potassium concentration is approximately 3–4 mM, the intracellular potassium concentration is around 130 mM. The intra- and extracellular concentrations of sodium are approximately 8–15 and 145 mM, respectively. Although the intracellular pH is often close to the extracellular pH (7.1–7.3), there is an inward electrochemical gradient for protons and bicarbonate, since their equilibrium potential is closer to 0 mV and cells have a negative resting membrane potential [9]. The steepest electrochemical gradient exists for calcium ions. The resting intracellular calcium concentration is lower than 100 nM, while the extracellular calcium concentration is around 2 mM. The intracellular chloride concentrations in mature neurones and astrocytes vary significantly. While the chloride concentrations in mature neurones are kept low (5–10 mM), they are between 30 and 60 mM in astrocytes, leading to a clearly more positive chloride equilibrium potential in astrocytes compared to neurones.

Ion movements and glial glutamate uptake

Neuronal activity leads to significant ion movements across the plasma membrane. These ionic fluxes are achieved not only by the opening of ionotropic transmitter receptors and ion channels, but also largely mediated by the activity of various ion transporters. The activation of the sodium-dependent glutamate transporter EAAT1 (glutamate transporter excitatory amino acid carrier 1) and 2 results in significant changes in intracellular sodium concentrations and pH in astrocytes (see [5]; see box. 1). The uptake of synaptically released glutamate in astrocytes is associated with the simultaneous uptake of three sodium ions and one proton in exchange for one potassium ion. The reversal potential for this electrogenic process lies heavily in the positive range (see box. 1). In this way, extracellular glutamate can be held at values under 1 µM, while glutamate concentration in astrocytes is in the millimolar range. Conversely, changes in concentration of the transported ions in turn influence the reversal potential or the driving force for glutamate uptake (see box. 1). Accordingly, it has been shown that un-

Abstract · Zusammenfassung

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Intracellular ion homeostasis. Impairment in hepatic encephalopathy

Abstract

Hepatic encephalopathy (HE) is a neuropsychiatric disorder associated with acute and chronic liver failure. Ammonium is the most likely toxin responsible for the observed neurological deficits, reaching 5 mM in the CNS during acute HE. Additionally, ammonium preferentially affects astrocytes. Conversion of glutamate to glutamine, occurring exclusively in astrocytes, is the sole pathway for ammonium detoxification, while glutamine increases in astrocytes during HE. Furthermore, elevated extracellular glutamate concentrations, commonly observed in HE patients, may result from reduced glutamate uptake into astrocytes. Finally, astrocytic swelling likely contributes to brain oedema, the predominant cause of mortality following acute liver failure. Alterations in intracellular ion concentrations may contribute to the observed cellular changes in HE. Ammonium causes intracellular acidifications, and increases intracellular calcium and sodium concentrations in astrocytes. In this review, we summarise current knowledge concerning ammonium-evoked changes in ion homeostasis, and discuss how such changes possibly contribute to the pathology of HE.

Keywords

Ammonium · Astrocyte · Neurone · Sodium · pH

Intrazelluläre Ionenhomeostase. Beeinträchtigung bei hepatischer Enzephalopathie

Zusammenfassung

Hepatische Enzephalopathie (HE) ist eine signifikante Begleiterscheinung akuter und chronischer Lebererkrankungen, die durch eine Vielzahl unterschiedlicher Funktionsstörungen des Gehirns gekennzeichnet ist. Als Hauptursache gilt ein Anstieg der Ammoniumionenkonzentration, wobei die HE primär als eine Gliafunktionsstörung angesehen wird. Ammonium wird durch das Enzym Glutaminsynthetase in Astrozyten mit Glutamat zu Glutamin fixiert, und Astrozyten weisen bei HE einen starken Anstieg ihres Glutamingehalts auf, während gleichzeitig eine Erhöhung der extrazellulären Glutamatkonzentration beobachtet wird. Schwellung der Astrozyten kann zu einem Hirnödem führen, das für die hohe Mortalitätsrate bei akutem Leberversagen verantwortlich ist. Einen bisher wenig beachteten Faktor bei der Entstehung der HE sind durch Ammonium induzierte Veränderungen der intrazellulären Ionenhomöostase. Neuere Untersuchungen zeigen, dass erhöhte Ammoniumkonzentrationen neben einer Ansäuerung auch Erhöhungen der Kalcium- und der Natriumkonzentration in Astrozyten hervorrufen. Im Übersichtsartikel möchten wir die durch Ammonium verursachten Ionenbewegungen beschreiben und diskutieren, in wieweit diese zur Pathologie der HE beitragen könnten.

Schlüsselwörter

Ammonium · Astrozyt · Neuron · Natrium · pH-Wert

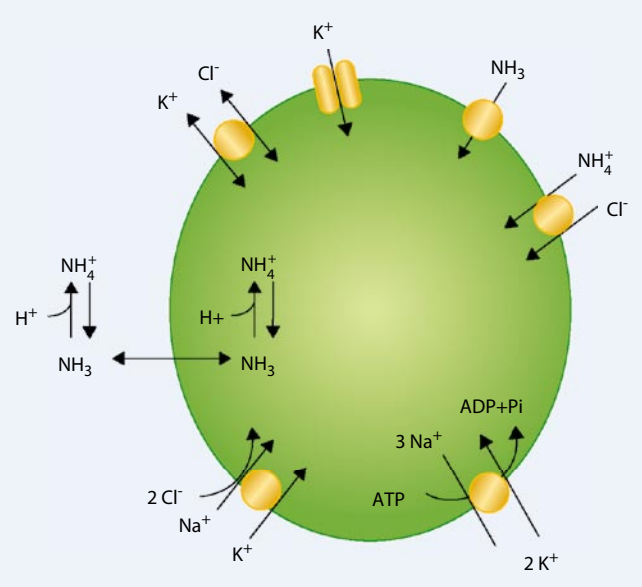

Fig. 2 ▲ Astrocyte ion regulation mechanisms and possible NH_4^+ influx pathways. Ammonia (NH_3) freely permeates the cell membrane. As a weak base, NH_3 consumes one proton in the cell increasing intracellular pH, i.e. an alkalinisation. Unlike ammonia, ammonium ions (NH_4^+) do not freely permeate the cell membrane. However, ammonium ions have a similar ionic diameter to potassium ions, and NH_4^+ crosses the cell membrane by substituting for potassium at ion channels. Moreover, NH_4^+ replaces potassium at various potassium-dependent transporters [sodium-potassium-chloride cotransport (NKCC), potassium-chloride cotransporter (KCC), Na^+/K^+-ATPase; see text]. In addition, evidence suggests that specific NH_4^+ transporters exist. The influx of NH_4^+ releases protons causing intracellular acidifications. In addition, NH_4^+ by altering the activity of ion transport mechanisms changes the homeostasis of other ions (e.g. sodium, potassium and chloride). Thus, in addition to pH changes, NH_4^+/NH_3-evoked changes in the intracellular concentrations of other ions may contribute to the toxic effects of NH_4^+/NH_3

der certain conditions, e.g. lack of oxygen, an increase in the intracellular sodium concentration can lead in extreme cases to a reversal in driving force for glutamate transport and can thus lead to outward glutamate transport.

Ammonium transport and ammonium-evoked pH changes

As discussed above, ammonium and ammonia play a central role in the development of HE. Therefore, the mechanism NH_4^+/NH_3 transport across the plasma membrane is of crucial importance to understanding the functional deficits observed during HE. In this regard, cell type-specific transport mechanisms may, at least in part, explain the cell type-specific effects of NH_4^+/NH_3.

Ammonia (NH_3) is a weak base, and as such exists in two forms depending on the pH; at physiological pH (approximately 7.2), 98% exists in the protonated form, i.e., as ammonium (NH_4^+). The unprotonated form, NH_3, freely permeates across cellular membranes and although NH_4^+ is impermeable, NH_3 within the cell associates with a proton forming NH_4^+ (**Fig. 1**). Thus, NH_3 influx and its equilibrium with NH_4^+ result in an intracellular gain of NH_4^+, which is dependent on the NH_4^+ electrochemical gradient [6].

The intracellular conversion of NH_3 to NH_4^+ consumes protons. Experimental addition of NH_4^+/NH_3, therefore, leads to initial intracellular alkalinisations in both neurones and astrocytes. Following the removal of NH_4^+/NH_3, NH_3 rapidly exists the cell, leaving protons behind in the cell resulting in a transient intracellular acidification (**Fig. 2 and Fig. 3**; [5]). Due to the ability of ammonium to change intracellular pH, the 'ammonium pre-pulse' technique is commonly used to investigate mechanisms of intracellular pH regulation and buffering. Cells are perfused for a few minutes with millimolar concentrations of NH_4^+/NH_3 (usually by adding ammonium chloride, NH_4Cl); the resultant changes in intracellular pH are then analysed under various test conditions.

Ammonium is also able to traverse the cell membrane. Ammonium is a known congener of potassium ions and due to the similar ionic properties (ionic diameter, hydration sheath) NH_4^+ may enter cells by substituting for potassium ions at ion channels and transporters (**Fig. 2**). In cultured neurones, for example, NH_4^+ enters through inwardly rectifying potassium channels. The influx of NH_4^+ releases protons causing intracellular acidifications. Cation-chloride cotransporters also contribute to NH_4^+ influx, and the differential expression of these transporters between neurones and glial cells may contribute to the differences in sensitivity of the cell types to NH_4^+. In cultured neurones, NH_4^+ uptake primarily involves potassium chloride cotransporters (KCC), whereas in cultured astrocytes the sodium-potassium-chloride cotransport (NKCC) may contribute [10].

A number of additional NH_4^+ transport pathways have been reported in various other cell types. For instance, bee retinal ganglion cells possess a dedicated NH_4^+ chloride cotransporter, while in some invertebrate neurones NH_4^+ influx is mediated by Na^+/K^+-ATPase [6].

The effects of ammonium-evoked changes in intracellular pH

One hallmark of HE is an increase in the extracellular glutamate concentration, a phenomenon consistently seen in HE patients and animal models (see [1, 7]). While reduced expression of glial glutamate transporters may contribute to the increased extracellular glutamate concentrations observed in chronic HE, other factors appear to be involved in acute HE. As described above (see **Fig. 2**), the application of NH_4^+/NH_3 leads to changes in intracellular pH, comprising an initial alkalinisations (NH_3 influx) followed by acidification (NH_4^+ influx). An article published in 2005 reported that transient, NH_4^+/NH_3-evoked alkalinisations releases calcium from intracellular stores, which

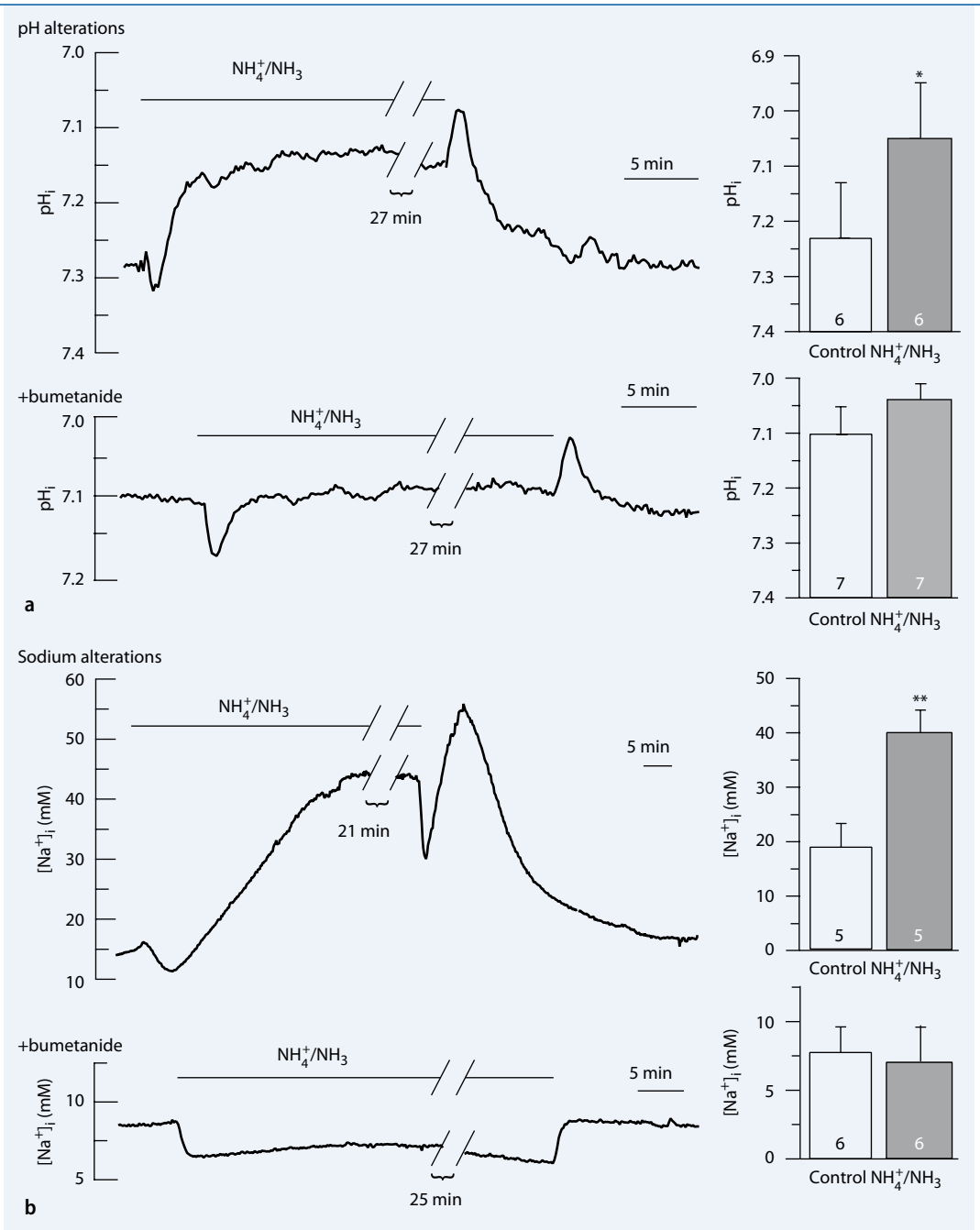

Fig. 3 ▶ Ammonium-evoked pH and sodium changes and the role of NKCC (sodium-potassium-chloride cotransport) in cultured hippocampal astrocytes. **a** Application of 5 mM NH_4^+/NH_3 caused a transient alkalinisation, followed by a sustained acidification. The pH was reduced by on average 0.19 units (see *right histogram* showing mean values with standard deviation). The presence of the NKCC blocker bumetanide prevented NH_4^+/NH_3-evoked acidifications (see *right histogram*). **b** NH_4^+/NH_3 caused a pronounced and sustained increase in the intracellular sodium concentration (*upper trace*), which was prevented in the presence of bumetanide (*lower trace*). Histograms on the right show mean values with standard deviation of several measurements

in turn mediated Ca^{2+}-dependent release of glutamate from cultured cortical astrocytes ([8]; see also [2]).

Ammonium-evoked changes in the intracellular sodium concentration

Investigations recently carried out in our laboratory focussed initially on the question of whether, in addition to intracellular pH, NH_4^+/NH_3 also influences intracellular sodium concentrations [5]. To this end, primary cultured rat hippocampal astrocytes were washed in saline solution containing 5 mM NH_4^+/NH_3 in exchange for sodium ions. Intracellular pH measurements employing the pH-sensitive fluorescent dye BCECF [2',7'-bis-(2-carboxyethyl)-5,6-carboxyfluorescein] showed that application of NH_4^+/NH_3 elicited transient alkalinisations within 1 min, as expected from the influx of NH_3. NH_4^+/NH_3-evoked alkalinisations were superseded by sustained acidifications with a mean value of pH 7.05 (**Fig. 3a;** [5]). Upon removal of NH_4^+/NH_3, transient acidifications were observed before pH returned to its original baseline value of around pH 7.25.

Quantitative determination of sodium concentration in cultured astrocytes using the sodium-sensitive fluorescent dye SBFI (see **Fig. 4**) shows that NH_4^+/NH_3 evoked pronounced increases in intracellular sodium concentration, in addition to the previously mentioned changes in intracellular pH. Indeed, NH_4^+/NH_3 increases sodium concentration from a baseline of around 18 mM to a mean value of 40 mM in astrocytes, and the elevated sodium concentration was maintained

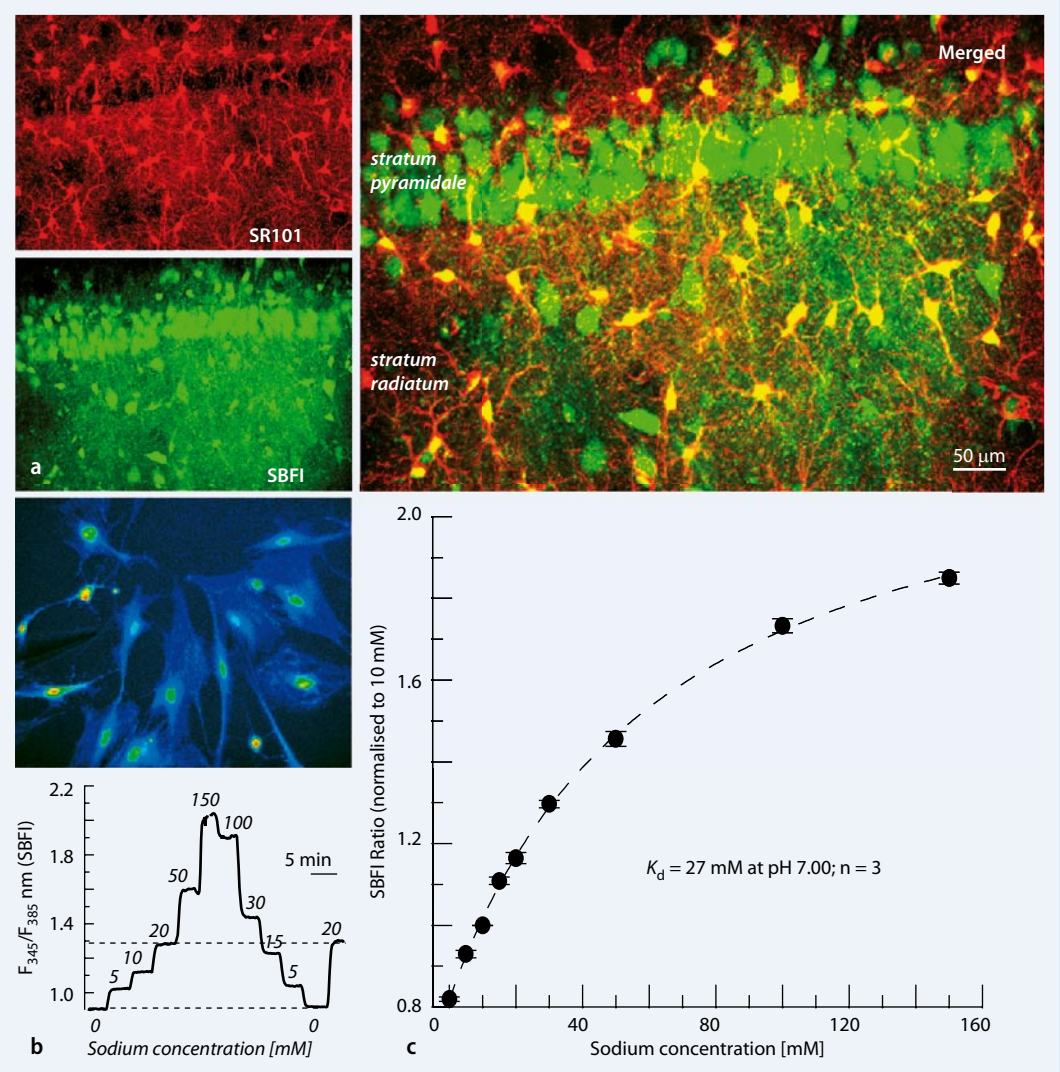

Fig. 4 ▲ Determination of intracellular ion concentrations using fluorescent dyes. **a** Ion-sensitive fluorescent dyes allow quantitative determination of intracellular ion changes using imaging techniques. Ejection of AM (acetomethyl-) esters of the sodium-sensitive dye SBFI-AM into a hippocampal brain slice using a glass pipette (technique according to Stosiek et al. 2003, PNAS 100) simultaneously loads astrocytes and neurones (*green channel*). The vital astrocyte marker SR101 was also employed (*red channel*), in order to easily differentiate between astrocytes and neurones (*merged image*; see [14, 15]). **b***Top* Cultured astrocytes loaded by incubation with SBFI-AM. *Bottom*: when loaded cells are rinsed with calibration solutions containing the sodium pore forming agent gramicidin, calibration of the fluorescent signal can be performed using ratiometric imaging (see [12]). Changes in sodium concentration cause reproducible changes in the fluorescence emission of SBFI. **c** Calibration curve summarising the results from several calibrations. Using calibration curves such as this, fluorescence values can be converted into absolute sodium concentrations

in the continued presence of NH_4^+/NH_3 (**Fig. 3b**). The NH_4^+/NH_3-evoked increases in intracellular sodium were reversible, with sodium concentrations returning to baseline following removal of NH_4^+/NH_3.

Inhibiting HCO_3^--dependent pH regulatory mechanisms using HCO_3^--free media increased the NH_4^+/NH_3-evoked acidifications and attenuated the sodium increases, suggesting that NH_4^+/NH_3 activates sodium-dependent intracellular pH regulatory mechanisms [5]. However, the NH_4^+/NH_3-evoked sodium increases were not simply a consequence of intracellular pH regulatory mechanisms. In fact, our investigations suggested that other sodium-dependent mechanisms of inward NH_4^+/NH_3 transport are involved. To examine the involvement of NKCC, we employed the specific blocker bumetanide. Inhibition of NKCC with bumetanide had prominent effects on NH_4^+/NH_3-evoked pH and sodium changes. Although the aforementioned mentioned transient alkalinisations were still observed following NH_4^+/NH_3 application, bumetanide prevented the sustained acidifications (**Fig. 3a**), as well as the pronounced increases in sodium concentration (**Fig. 3b**).

Our results clearly show that NKCC activation by NH_4^+/NH_3 is the basic

mechanism responsible for both the acidifications and the large increases in sodium concentration observed in cultured astrocytes. Moreover, NKCC appears to be the main mechanism of inward NH_4^+ transport in cultured astrocytes, findings which are in agreement with those published in 2006 by Titz et al. [10], suggesting the involvement of NKCC in NH_4^+ influx [10].

In neurones, the mechanism of NH_4^+ influx probably depends on maturity or degree of differentiation. The level of NKCC expression is high in many immature neurones (postnatal day 0–10), dropping sharply thereafter. Therefore, NH_4^+ influx in mature neurones probably occurs via alternative mechanisms. Published data [10] indicate the involvement of KCC in the NH_4^+ influx into cultured neurones and supports the notion that NH_4^+ influx pathways in neurones and astrocytes are different. However, the pathways for NH_4^+ influx into neurones and astrocytes *in situ* are as yet unknown.

Ammonium-evoked ion shifts and changes in glial glutamate uptake

The observation that NH_4^+/NH_3 evokes changes in both intracellular pH and sodium concentrations raises the possibility that NH_4^+/NH_3 may also reduce sodium-dependent glutamate uptake in astrocytes by reducing the inwardly directed proton and sodium gradients (see Box. 1). This could be relevant to the pathology of HE, during which elevated levels of extracellular glutamate concentrations are consistently observed.

To investigate this possibility, we selectively activated glial glutamate transporters with bath applications of the transportable agonist D-aspartate. Brief applications of D-aspartate caused transient intracellular acidifications, as well as transient sodium increases in astrocytes, reflecting glutamate transport activity (see Box. 1; **Fig. 5a**; [5]). In the presence of NH_4^+/NH_3, the amplitude of D-aspartate-induced acidifications and sodium increases were reduced by 80%–90%, suggesting a strong reduction in glial glutamate uptake (**Fig. 5a**).

As reported above, inhibition of NKCC using bumetanide prevents $NH_4^+/$

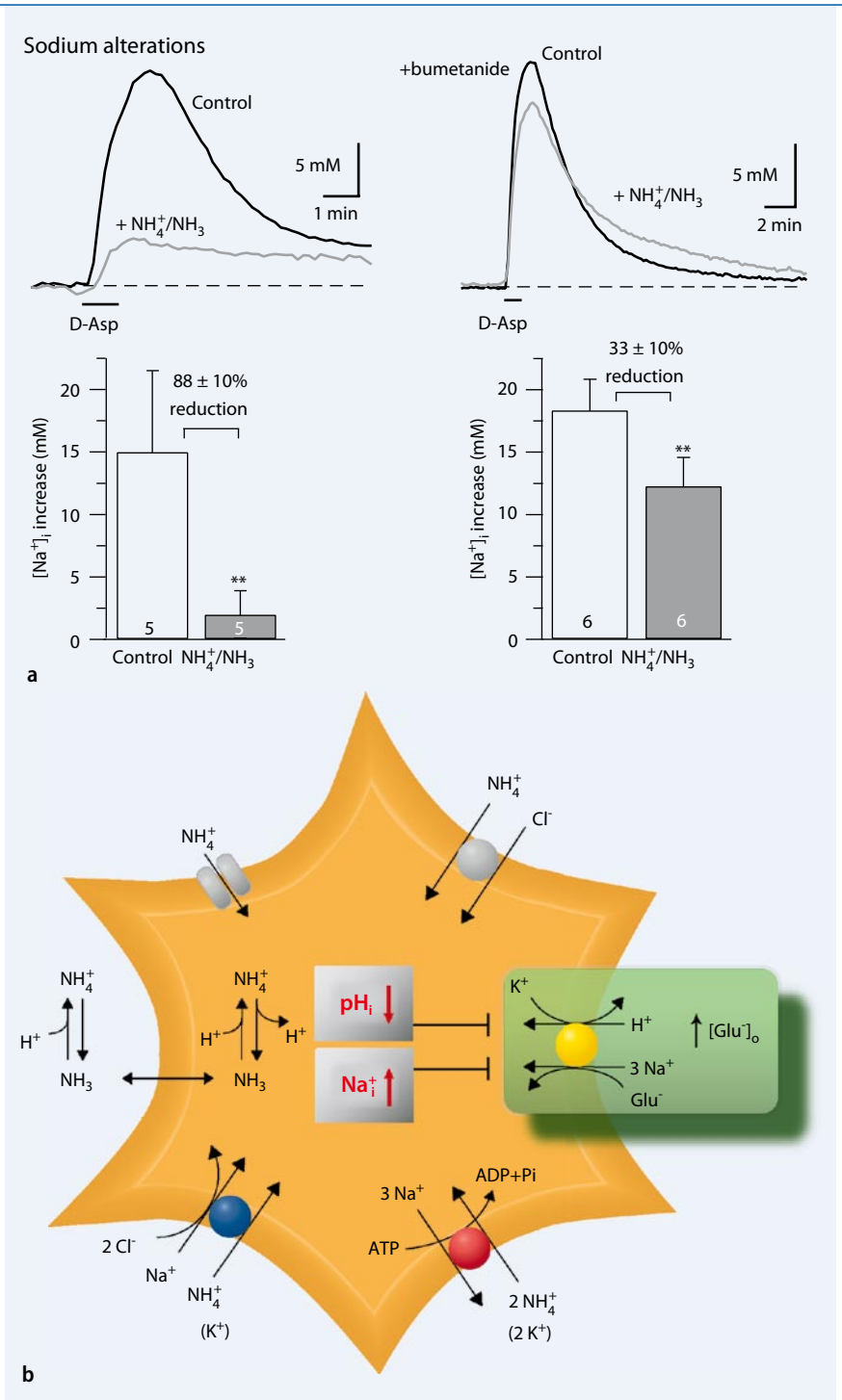

Fig. 5 ▲ Ammonium-evoked ion changes reduce glutamate uptake in cultured hippocampal astrocytes. **a** Application of D-aspartate (1 min, 1 mM) activates glutamate transporters causing increases in sodium (*black trace*). In the presence of 5 mM NH_4^+/NH_3 (*grey trace*), D-aspartate-induced sodium increases were reduced by almost 90%. *Right*: The presence of the NKCC blocker bumetanide attenuated the ability of NH_4^+/NH_3 to reduce D-aspartate-induced sodium increases. *Lower panel*: histograms showing the mean values and standard deviation of several measurements. **b** A schematic summarising the proposed mechanisms of ammonium-evoked ion changes and the effects on glial glutamate uptake. Ammonium is transported to the cells primarily via NKCC. The resultant acidifications and increases in sodium concentration significantly reduces the driving force for glutamate uptake. (Modified from [5], with the kind permission of Wiley-Liss)

NH_3-evoked acidifications and increases in sodium concentration in astrocytes (see **Fig. 2**). We, therefore, investigated the effect of bumetanide on the NH_4^+/NH_3-evoked inhibition of glial glutamate uptake.

Application of bumetanide alone did not significantly alter the amplitude, nor the time course of D-aspartate-induced pH and sodium transients in astrocytes in the absence of NH_4^+/NH_3. Bumetanide, however, significantly attenuated the ability of NH_4^+/NH_3 to reduce D-aspartate-induced pH and sodium transients (**Fig. 5a**). The results suggest that NH_4^+/NH_3-evoked acidifications and increases in intracellular sodium concentration strongly reduce the driving force for glial glutamate uptake (**Fig. 5b**). Preventing NH_4^+/NH_3-evoked acidifications and increases in intracellular sodium alleviated the NH_4^+/NH_3-evoked reduction in glutamate transport activity. The proportion of glutamate transport activity inhibited as a result of NH_4^+/NH_3-evoked changes in pH and intracellular sodium concentration was approximately 50%, and corresponds to that predicted by decreases in the electrochemical gradients of protons and sodium ions (see box. 1; [5]).

The role of ammonium-induced ion changes in HE

The transient alkalinisations and subsequent calcium-dependent release of glutamate from astrocytes upon NH_4^+/NH_3 application as mentioned above could represent crucial first steps contributing to an increase in the extracellular glutamate concentration [4]. It has also been reported that NH_4^+-mediated calcium increases cause tyrosine nitration of various proteins in astrocytes, which may be of relevance in HE [4].

Linking NH_4^+ influx to ion transport mechanisms also causes permanent changes in intracellular ion homeostasis, which may contribute to the development of HE. Moreover, changes in intracellular ion homeostasis maintained in the prolonged presence of NH_4^+/NH_3 are likely of greater clinical relevance than transient ion shifts lasting only several minutes. Whereas sodium-independent transport (KCC) mediates NH_4^+ influx in cultured neurones, sodium-dependent transport (NKCC) is involved in NH_4^+ influx in astrocytes. The strong influx of sodium ions (as well as co-transported potassium and chloride ions) following NKCC activation contributes in particular to the astrocyte swelling observed during acute HE. This is significant since cerebral oedema is the most frequent cause of death following acute liver failure [1].

In this context, our data also show that glial glutamate uptake was strongly reduced by a reduction in the inward driving force within 30 min after initiation of NH_4^+/NH_3 application. This time frame corresponds to the increase observed in the extracellular glutamate concentration in animals under acute NH_4^+/NH_3 exposure, and is likely too rapid to reflect changes in the expression level of glutamate transporters.

NH_4^+/NH_3-evoked changes in intracellular pH may also contribute to other aspects of HE. Intracellular pH is a known modulator of cellular function and influences enzyme activity, as well as the activity of ion channels and transport mechanisms. The steady-state intracellular acidifications observed following prolonged NH_4^+/NH_3 exposure are similar in magnitude to those observed, for instance, during anoxia. Acidifications generally result in inhibition of neuronal excitability and enzyme activity [9]. Moreover, a reduction in pH inhibits the release of calcium from intracellular stores, an important intracellular signalling mechanism in both astrocytes and neurones. General inhibition of neuronal excitability as well as a reduction in intracellular calcium release may contribute to the deficits in neurotransmission observed in HE patients.

While many fundamental questions regarding the development of HE remain unanswered, the studies carried out to date clearly show that ammonium-evoked ion fluxes may contribute in a causal manner to cellular and, in particular, glial dysfunction in HE. Further elucidation of the underlying cellular mechanisms will hopefully contribute to the development of better therapeutic possibilities for this disease in the future.

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References

1. Felipo V, Butterworth RF (2002) Neurobiology of ammonia. Progress Neurobiology 67:259–279
2. Görg B, Morwinsky A, Keitel V, Schrör K, Häussinger, D (2010) Ammonia triggers exocytotic release of L-glutamate from cultured rat astrocytes. Glia 58:691–705
3. Häussinger D, Kircheis G, Fischer R, Schliess F, vom Dahl S (2000) Hepatic encephalopathy in chronic liver disease: a clinical manifestation of astrocyte swelling and low-grade cerebral edema? J Hepatol 32:1035–1038
4. Häussinger D, Görg B (2010) Interaction of oxidative stress, astrocyte swelling and cerebral ammonia toxicity. Curr Opin Clin Nutr Metab Care 13:87–92
5. Kelly T, Kafitz KW, Roderigo C, Rose CR (2009) Ammonium-evoked alterations in intracellular sodium and pH reduce glial glutamate transport activity. Glia 57:921–934

6. Marcaggi P, Coles J (2001) Ammonium in nervous tissue: transport across cell membranes, fluxes from neurons to glial cells, and role in signalling. Progress Neurobiology 64:157–183
7. Rose C (2002) Increased extracellular brain glutamate in acute liver failure: Decreased uptake or increased release? Metabolic Brain Disease 17:251–261
8. Rose C, Kresse W, Kettenmann H (2005) Acute insult of ammonia leads to calcium-dependent glutamate release from cultured astrocytes, an effect of pH. J Biol Chem 280:20937–20944
9. Deitmer JW, Rose CR (1996) pH regulation and proton signalling by glial cells. Progress in Neurobiology 48:73–103
10. Titz S, Hormuzdi S, Lewen A, Monyer H, Misgeld U (2006) Intracellular acidification in neurons induced by ammonium depends on KCC2 function. Eur J Neuroscience 23:454–464
11. Weissenborn K, Bokemeyer M, Krause J, Ennen J, Ahl B (2005) Neurological and neuropsychiatric syndromes associated with liver disease. AIDS 19: s93–s98
12. Rose CR, Ransom BR (1996) Intracellular sodium homeostasis in rat hippocampal astrocytes. J Physiol 491: 291-305
13. Stosiek C, Garaschuk O, Holthoff K, Konnerth A (2003) In vivio two-photon calcium imaging of neuronal networks. PNAS 100: 7319-7324
14. Nimmerjahn, Kirchhoff F, Kerr JN, Helmchen F (2004) Sulforhodamine 101 as a specific marker of astroglia in the neocortex in vivo. Nature Methods 1: 31-37
15. Kafitz KW, Meier SD, Stephan J, Rose CR (2008) Develpomental profile and properties of sulforhodamine 101-labeled glial cells in acute brain slices of rat hippocampus. J Neurosci Methods 169: 84-92
16. Zerangue N, Kavanaugh MP (1996) Flux coupling in neuronal glutamate transporter. Nature 383: 634-637

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