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ORIGINAL PAPER

Handling of Copper and Copper Oxide Nanoparticles by Astrocytes

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Abstract Copper is an essential trace element for many important cellular functions. However, excess of copper can impair cellular functions by copper-induced oxidative stress. In brain, astrocytes are considered to play a prominent role in the copper homeostasis. In this short review we summarise the current knowledge on the molecular mechanisms which are involved in the handling of copper by astrocytes. Cultured astrocytes efficiently take up copper ions predominantly by the copper transporter Ctr1 and the divalent metal transporter DMT1. In addition, copper oxide nanoparticles are rapidly accumulated by astrocytes via endocytosis. Cultured astrocytes tolerate moderate increases in intracellular copper contents very well. However, if a given threshold of cellular copper content is exceeded after exposure to copper, accelerated production of reactive oxygen species and compromised cell viability are observed. Upon exposure to sub-toxic concentrations of copper ions or copper oxide nanoparticles, astrocytes increase their copper storage capacity by upregulating the cellular contents of glutathione and metallothioneins. In addition, cultured astrocytes have the capacity to export copper ions which is likely to involve the copper ATPase 7A. The ability of astrocytes to efficiently accumulate, store and export copper ions suggests that astrocytes have a key role in the distribution of copper in brain. Impairment of this astrocytic function may be involved in diseases which are connected with disturbances in brain copper metabolism.

Keywords Astrocytes · Copper · GSH · Metallothioneins · Oxidative stress · Transport

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Introduction

Copper is an essential trace element which serves as cofactor or structural component in a large number of enzymes [1–3]. Important copper-containing enzymes are cytochrome c oxidase, copper-containing superoxide dismutases and ceruloplasmin which connect copper to processes such as mitochondrial energy production, antioxidative defence and iron metabolism, respectively [3–5]. However, excess of copper can harm cells due to its potential to inactivate protein functions and to catalyse hydroxyl radical formation by a Fenton-like reaction, thereby inducing oxidative stress and cell damage [3, 6, 7].

In the brain, astrocytes have important functions in the modulation of synaptic behavior [8–12], but also in energy metabolism [13, 14], in neurotransmitter metabolism [15], and in the defence against oxidative stress and toxins [12, 16, 17]. Due to the role of astrocytes in many important aspects of normal brain physiology, compromised astrocyte functions have been proposed to be involved in various pathological processes and in the etiology of many neurodegenerative disorders including Alzheimer's disease, Parkinson's disease and Menkes disease [11, 18, 19].

Astrocytes are considered to play a crucial role in the brain copper homeostasis. Histochemical analysis of the brain revealed high copper contents in astrocytes [20–22]. Also the elevated brain copper levels in the North

Ronaldsay sheep, an animal model for copper toxicosis, have been connected with an increased astrocytic copper accumulation and with a strong astrocytic immunoreactivity for the metal storing metallothioneins (MTs) [23]. Cultured astrocytes contain a basal copper content of 1–2 nmol/mg protein [24–26], but this content is rapidly increased after exposure of the cells to copper in low molecular weight form [24, 26–32] or in nanoparticular form [24, 33], demonstrating that astrocytes express efficient transport systems for the uptake of copper. In addition, astrocytes improve their copper storage capacity after exposure to copper by increasing cellular glutathione (GSH) and MT levels [2, 28, 33], and are able to release copper ions [30].

Recently copper oxide nanoparticles (CuO-NPs) gained a lot of interest due to their catalytic, physical and biocidal properties. CuO-NPs are used on a large scale as additives for products such as paints, wood preservatives, lubricants, heat-transfer fluids, sensors, conductive films, plastics and solid propellants [34–38]. In addition, CuO-NPs are used for medical applications in water filters, bandages and for sterile surface coatings [39–41]. For several cell types, CuO-NPs are known to have toxic potential [35, 39, 42] and among various types of metal-containing nanoparticles, CuO-NPs have been shown to be the most toxic one [43]. As inhalation is a major route for internalisation of nanoparticles in humans [44], the risk of an occupational exposure to CuO-NPs is increased in an environment where high amounts of this material are generated and released into the air, especially during welding and during the operation of electric motors [45, 46]. Inhaled nanoparticles can enter the brain directly by the olfactory bulb or indirectly by crossing the blood–brain barrier after entry in the bloodstream via the lungs [47–51]. Although injection of CuO-NPs in rats has been reported to compromise learning and memory and to impair hippocampal long-term potentiation [52], little is known on the uptake and metabolism of CuO-NPs in brain cells. Only very recently, some consequences of an exposure of cultured astrocytes to CuO-NPs have been reported [24, 33].

This article gives an overview on the current knowledge on the uptake and metabolism of copper and copper oxide nanoparticles in astrocytes. In addition, copper-induced toxicity and copper-induced alterations in the basic glucose and GSH metabolism of astrocytes will be summarised.

Uptake of Copper

Uptake of Low Molecular Weight Copper

Astrocytes accumulate copper ions efficiently in vivo [23, 53] and in vitro [26–31, 54]. Copper accumulation by cultured astrocytes is saturable, has a low K_M value of about 10 μM for copper ions and depends strongly on the incubation temperature [26]. The copper transporter Ctr1 appears to be involved in copper uptake in cultured astrocytes (Fig. 1), as previously shown for peripheral cells [55–57]. This view is supported by the expression of Ctr1 in cultured astrocytes [26, 58]. However, to which extent Ctr1 and/or other transporters contribute to astrocytic copper uptake has not been reported so far.

Presence of zinc ions has been reported to inhibit copper accumulation in astrocytes [26, 27], suggesting that a Ctr1-independent transport mechanism is also involved in astrocytic copper uptake. The divalent metal transporter DMT1, which mediates a proton-coupled co-transport of various types of metal ions across membranes [59], is a likely candidate for Ctr1-independent copper uptake in astrocytes (Fig. 1). At least, this transporter is expressed in astrocytes in vitro [60, 61] and in vivo [62]. Consistent with a contribution of DMT1 in the copper uptake into astrocytes is the pH-dependency of astrocytic copper accumulation and the inhibition of copper accumulation by other DMT1 substrates such as manganese and cadmium ions [26]. Another protein which has been suggested to contribute to copper uptake by astrocytes is the prion protein which is considered as a receptor for cellular uptake or efflux of copper [63, 64]. However, the potential role of prion protein in astrocytic copper metabolism is still under debate [2].

Both the copper transporters Ctr1 and DMT1 have been reported to transport Cu^+ rather than Cu^{2+} [57, 65]. As cultured astrocytes accumulate copper quickly after exposure to Cu(II) salts [26–31, 54], an ecto-cuprireductase located in the plasma membrane has been suggested to be involved in astrocytic copper uptake [3] (Fig. 1). One metal-reductase involved could be Dcyt*b,* a cytochrome *b* family homologue, which has been shown to be expressed in cultured astrocytes [60, 66]. Astrocytes also contain the mRNAs of proteins of the 6-transmembrane epithelial antigen of prostate (Steap) family, suggesting that astrocytes may also contain the respective proteins which have extracellular cuprireductase activity [67–69].

Presence of extracellular ascorbate strongly accelerates copper uptake in cultured astrocytes [26, 32], suggesting that the reduction of Cu^{2+} to Cu^+ is the rate-limiting step in copper uptake by astrocytes. In brain, extracellular ascorbate concentrations are in the concentration range of 200–400 μM [70, 71] and a continuous reduction of Cu^{2+} by ascorbate may facilitate copper uptake into brain cells. As cultured astrocytes have been reported to release ascorbate [72, 73] these cells may also in brain provide the reducing compound required to generate Cu^+ for uptake into cells. In addition to ascorbate, also α-tocopherol has the potential to reduce Cu^{2+} to Cu^+ and to accelerate copper accumulation in cultured astrocytes [32].

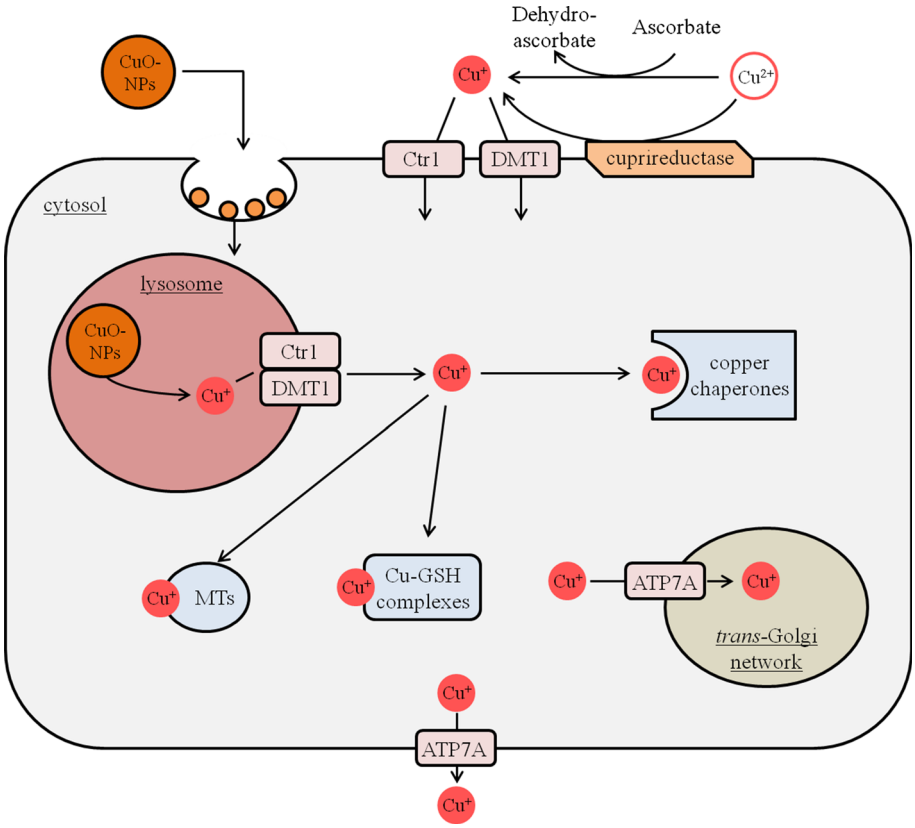

Fig. 1 Uptake and metabolism of copper ions and CuO-NPs in astrocytes. Astrocytes predominantly take up ionic copper by the copper transporter Ctr1 and the divalent metal transporter 1 (DMT1). Extracellular reduction of Cu^{2+} to Cu^{+} is catalysed by ectocuprireductases or depends on the presence of reducing substances such as ascorbate. Endocytotic mechanisms are responsible for the uptake of CuO-NPs and will direct the internalised particles to the lysosomal compartment where copper ions are liberated and exported via Ctr1 or DMT1 into the cytosol. Excess of cytosolic copper can be stored in metallothioneins (MTs) or as complex with glutathione (GSH). Copper chaperones transport copper for incorporation to their specific cellular target enzymes. The copper transport into the trans-Golgi network and the export of copper from astrocytes are mediated by the copper ATPase ATP7A

Components of the incubation media strongly affect the uptake and potential adverse effects of copper in cultured astrocytes. While cultured astrocytes accumulate copper efficiently from amino acid-free buffers [26, 27], copper uptake is much slower in histidine-containing buffer [54], in complex cell culture media [28–30] or in the presence of serum [24]. Reason for these differences in the astrocytic copper accumulation is most likely the efficient binding of copper ions by histidine, serum proteins and/or other components of complex media which limit the availability of the copper species needed for cellular copper import.

Uptake of Copper Oxide Nanoparticles

Exposure of cultured astrocytes with CuO-NPs in serum-containing medium caused a time- and concentration-dependent accumulation of copper [24, 33]. This uptake of CuO-NPs is likely to involve endocytotic processes (Fig. 1), as previously reported for the astrocytic uptake of other types of metal-containing nanoparticles. At least for iron oxide nanoparticles and silver nanoparticles was shown that clathrin-mediated endocytosis and macropinocytosis contribute to astrocytic nanoparticle accumulation [74, 75]. As inhibitors of clathrin-mediated endocytosis and macropinocytosis lower also the cellular accumulation of CuO-NPs by cultured astrocytes (data not shown), these endocytotic pathways are likely to be involved in astrocytic CuO-NP uptake.

The uptake of CuO-NPs in cultured astrocytes is strongly affected by lowering the temperature. Compared to an incubation at 37 °C, the accumulation of CuO-NPs by astrocytes is lowered at an incubation temperature of 4 °C by around 50 % [24], as previously also shown for the astrocytic uptake of other types of metal containing nanoparticles [74–78]. The amount of copper determined after incubation of astrocytes with CuO-NPs at 4 °C is likely to represent predominately particles which are extracellularly attached to the plasma membrane, as previously demonstrated for iron oxide nanoparticles [76, 77].

Thus, the difference in specific cellular copper contents of astrocytes treated with CuO-NPs at 37 and 4 °C can be considered to represent the copper which has been internalised by the cells. Direct comparison of the cellular copper accumulation in astrocytes after incubation with identical amounts of copper in low molecular weight ($CuCl_2$) or particle (CuO-NP) form revealed that, although CuO-NP-treated astrocytes contain higher specific copper contents (attached plus internalised material) compared to $CuCl_2$-treated cells, the amount of internalised copper was similar after treatments with equal concentrations of copper as CuO-NPs or $CuCl_2$ [24].

Astrocytic Toxicity Induced by Application of Copper Salts or Copper Oxide Nanoparticles

Although cultured astrocytes efficiently accumulate copper, these cells have been reported to be rather resistant against toxicity induced by low or moderate concentrations of copper salts [26, 79, 80] (Table 1), while the viability of cultured neurons is already compromised by exposure to low micromolar concentrations of copper [80]. For cultured astrocytes, even a five-fold increased in the specific cellular copper content does not acutely compromise the viability [24, 26, 27, 33]. However, as soon as a threshold level of around 10 nmol copper/mg protein is exceeded by a given exposure of cultured astrocytes to low molecular weight copper or copper oxide nanoparticles, substantial toxicity is observed (Fig. 2). The high cellular GSH content and the high antioxidative potential of astrocytes [16, 81, 82] are likely to contribute substantially to the resistance of astrocytes against exposure to moderate concentrations of copper. This view is strongly supported by the increased vulnerability against copper-mediated toxicity of astrocytes that had been treated with buthionine sulfoximine to lower the cellular GSH concentration [83].

Copper toxicity depends strongly on the composition of the medium used for the exposure which strongly influences cellular copper uptake. For example, presence of proteins or copper chelators in the medium lowers both the cellular accumulation of copper and the copper-induced toxicity [24, 26, 32], while copper reducing compounds in the medium accelerate astrocytic copper uptake and thereby increase toxicity [32].

In contrast to iron oxide nanoparticles and silver nanoparticles which even in concentrations of up to 4 and 0.3 mM, respectively, did not acutely compromise the viability of cultured astrocytes [75, 76, 78, 84], in serum-containing medium already concentrations above 100 μM of copper in form of $CuCl_2$ or CuO-NPs have a severe toxic potential on astrocytes [24, 33]. As similar amounts of copper are internalised by astrocytes exposed to CuO-NPs or $CuCl_2$ [24], the toxic potential of the CuO-NPs is likely caused by copper ions released from the internalised NPs. This view is strongly supported by the protection of astrocytes against CuO-NP-induced toxicity in presence of the membrane-permeable copper chelator tetrathiomolybdate (TTM) [24].

The observed toxicity of an excess of cellular copper is most likely the consequence of several adverse processes. Copper ions inactivate cellular oxidoreductases, for example lactate dehydrogenase [85–88] (Table 1), suggesting that an excess of copper in cells will impair cellular energy metabolism. In addition, excess of copper ions facilitates the production of reactive oxygen species (ROS) by a Fenton-like reaction [3]. Indeed, evidence of increased ROS production [24, 32, 80, 89] and lipid peroxidation [26, 90] was reported for astrocytes that had been treated with copper ions or CuO-NPs (Table 2). Thus, copper-induced oxidative stress is likely to be a major component contributing to the toxic potential of high concentrations of various copper sources on cultured astrocytes (Fig. 3; Table 2).

Intracellular Trafficking and Storage of Copper and Copper Oxide Nanoparticles in Astrocytes

Uptake of copper ions by transporters in the plasma membrane will directly increase the cytosolic copper content of astrocytes, while copper ions have to be liberated first from internalised CuO-NPs to access the astrocytic copper metabolism. This liberation of copper ions from CuO-NPs occurs most likely in astrocytic lysosomes. Although details on the intracellular trafficking of internalised CuO-NPs have not been reported so far, it appears likely that these particles will have a similar intracellular fate in astrocytes as other types of metal-containing NPs [76, 91–93]. After endocytotic uptake, CuO-NPs will most likely be directed in intracellular vesicles to the lysosomal compartment. Lysosomes contain an acidic and reducing environment [94, 95] which facilitates the liberation of copper ions from CuO-NPs [96]. In addition, these conditions will provide Cu^+ ions which are transported from the lysosomes into the cytosol. Responsible for this transport are most likely DMT1 and Ctr1 as both transporters have been reported to be present in astrocytic lysosomes [97, 98] (Fig. 1). Thus, copper liberated from internalised CuO-NPs in lysosomes will increase the cytosolic copper pool and will enter the normal astrocytic copper metabolism.

Excess of cytosolic copper in astrocytes will be shuttled by copper chaperones to their specific targets and will be sequestered by GSH and/or stored in MTs [2, 3]. These processes are highly efficient and it has been discussed that under physiological conditions less than one free copper

Table 1 Consequences of an exposure of cultured astrocytes with copper salts

Altered cellular parameter	Time of exposure (h)	Applied concentration (μM)	Presence of serum	References
Lowered MTT reduction	3	300	+	[24]
	6	1000	+	[32]
	4	10	−	[27]
	24	100	+	[79]
Decreased cellular LDH activity	4	30	−	[27]
	24	75	−	[29]
	16	100	−	[29]
Lowered neutral red retention	16	75	−	[29]
Increased appearance of pyknotic nuclei	48	20	+	[80]
Impaired membrane integrity	2	30	−	[27]
	6	1000	+	[32]
Accelerated generation of ROS	3	1000	−	[32]
Decreased ATP levels	8	20	+	[89]
Induction of mitochondrial permeability	24	20	+	[80]
Increased tyrosine nitration of proteins	24	20	+	[80]
Decreased G6PDH activity	4	10	−	[27]
Decreased GR activity	4	3	−	[27]
PDH inactivation	6	20	−	[89]
Activation of caspase-3	6	20	−	[89]
Reduced Fe, Mn and Zn accumulation	24	150	+	[31]
Accelerated GSH export	3	30	−	[27]
	24	10	−	[28]
Increased cellular GSH content	3	30	−	[28]
Increased cellular GSSG content	3	30	−	[27]
Stimulated glycolytic flux	8	30	−	[29]
Translocation of ATP7A	8	30	−	[30]
Increased metallothionein expression	24	30	−	[2]
	168	10	+	[54]
Increased ceruloplasmin expression	168	10	+	[54]
Increased Hspa5 expression	24	150	+	[31]
Increased prion protein expression	18	50	+	[54]
	48	1	+	[54]

The table summarises the reported consequences of an exposure of cultured primary astrocytes to copper salts that had been applied in the concentration listed for the indicated time period in either serum-free or serum-containing medium

G6PDH glucose-6-phosphate dehydrogenase, *GR* glutathione reductase, *GSSG* glutathione disulfide, *MTT* methylthiazolyldiphenyl-tetrazolium bromide, *PDH* pyruvate dehydrogenase

ion is present per cell [99, 100]. Copper chaperones rapidly bind cytosolic Cu^{+}-ions to shuttle them to their designated cellular targets [100]. The mRNAs for the copper chaperones Cox17, copper chaperone for superoxide dismutase (CCS) and antioxidant protein 1 (Atox1) are present in isolated mouse astrocytes [69], suggesting that also the respective proteins are produced in astrocytes and contribute to cellular copper distribution. Cox 17 transfers copper to the cytochrome c oxidase in the inner mitochondrial membrane, CCS transfers the copper to the cytosolic Cu/Zn superoxide dismutase and Atox1 shuttles the copper to the copper export protein ATP7A which is located in the trans-Golgi-network [100].

Cultured astrocytes contain high cytosolic concentrations of GSH [16, 82, 101] and express several types of MTs [102–104]. As astrocytes have the capacity to increase their cellular GSH content after exposure to metal ions [16, 17] and to upregulate their MT levels [103, 105, 106], these cells can improve their storage capacity for metals. This is also the case for copper-treated astrocytes. Copper can be stored as Cu(I)-complex with GSH or MTs [107, 108]. Exposure of cultured astrocytes to $CuCl_2$ or to CuO-NPs

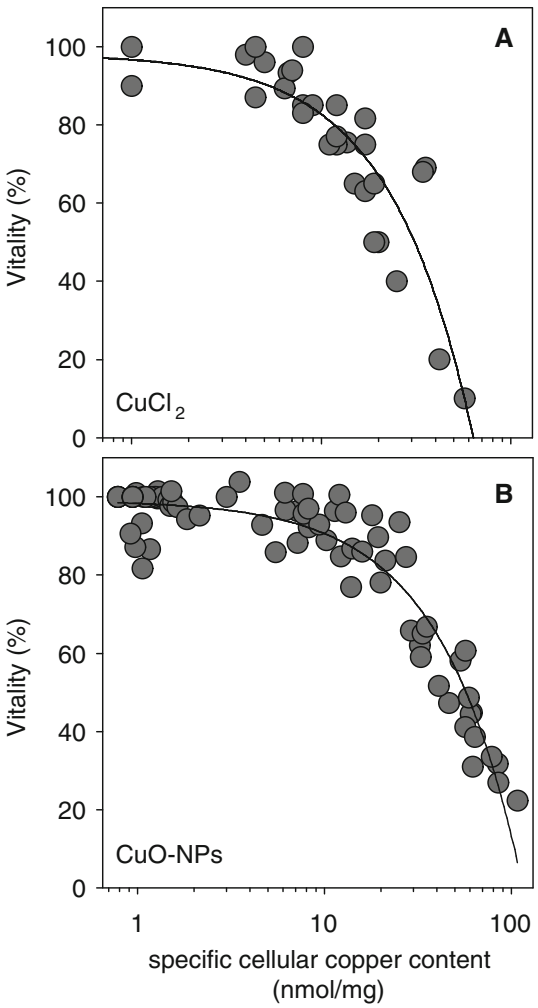

Fig. 2 Correlation of the specific cellular copper content with the toxicity of cultured astrocytes. The specific cellular copper contents of cultured astrocytes after incubation with $CuCl_2$ (**a**) or CuO-NPs (**b**) in various media and for various incubation periods were correlated with the reduction in cellular vitality reported for the respective condition by determining the MTT reduction capacity, LDH release or the neutral red retention capacity. The experimental data for this analysis were taken from [24, 27, 29, 33]

increases the cellular GSH concentration [28, 33] and the specific cellular MT levels [2, 33]. These adaptive responses increase the storage capacity of astrocytes for copper and are likely to contribute to the high resistance of astrocytes against moderate concentrations of copper-containing substances.

Export of Copper from Astrocytes

Cultured astrocytes release copper in a time-, concentration- and temperature-dependent manner [30]. Transporters involved in copper export from mammalian cells are the copper ATPases ATP7A and ATP7B [3, 109, 110]. Both

Table 2 Comparison of the effects of $CuCl_2$ or CuO-NPs on the vitality and the metabolism of cultured astrocytes

Altered cellular parameter	$CuCl_2$	CuO-NPs
Lowered MTT reduction	[24, 27, 79]	[24, 33]
Impaired membrane integrity	[27, 32]	[24, 33]
Generation of ROS	[32]	[24]
Decreased cellular LDH activity	[27, 29]	[24]
Stimulated glycolytic flux	[29]	[33]
Increased cellular GSH content	[28]	[33]
Accelerated GSH export	[27, 28]	[24]
Increased metallothionein levels	[2, 54]	[24]

The table lists references which report consequences of an exposure of cultured primary astrocytes to either $CuCl_2$ or CuO-NPs

transporters are expressed in brain cells [109]. Astrocytes predominantly express ATP7A but also contain the mRNA of ATP7B [30, 69, 109]. In brain, the expression levels of ATP7B are low [109], supporting the view that ATP7A is predominately responsible for copper export from astrocytes [30, 109].

ATP7A is localised predominately at the *trans*-Golgi network if cells contain low cellular copper concentrations, but is reversibly translocated to a vesicular compartment in close proximity to the plasma membrane when cytosolic copper levels rise [30, 109, 110]. This copper-dependent reversible translocation of ATP7A between two compartments has also been reported for cultured astrocytes [30]. In addition, astrocytes in the brindled mouse model of Menkes disease, a disease caused by mutated ATP7A which impairs the translocation of ATP7A to the plasma membrane, strongly accumulate copper [20, 21, 111, 112]. Recently the heat shock 70 kDa protein 5 (Hspa5) has been suggested to be involved in the copper-dependent trafficking of ATP7A in astrocytes [31]. Consistent with such a function, the expression of Hspa5 is strongly induced by copper [31].

The demonstration that cultured astrocytes are able to export copper [30] provides first experimental evidence which supports the hypothesis that astrocytes would have the potential to supply other brain cells with copper [20–22]. This copper export ability of astrocytes is a crucial property which defines astrocytes as a key player in the copper homeostasis of the brain [2, 3, 113, 114].

Copper Exposure Modulates the Glucose and GSH Metabolism of Astrocytes

Exposure of cultured astrocytes to subtoxic concentrations of $CuCl_2$ [29] or CuO-NPs [33] caused an acceleration of glycolytic flux as demonstrated by an increased glucose consumption and lactate release. The observed acceleration of glycolytic flux was not due to mitochondrial impairment but was prevented by inhibition of protein synthesis [29],

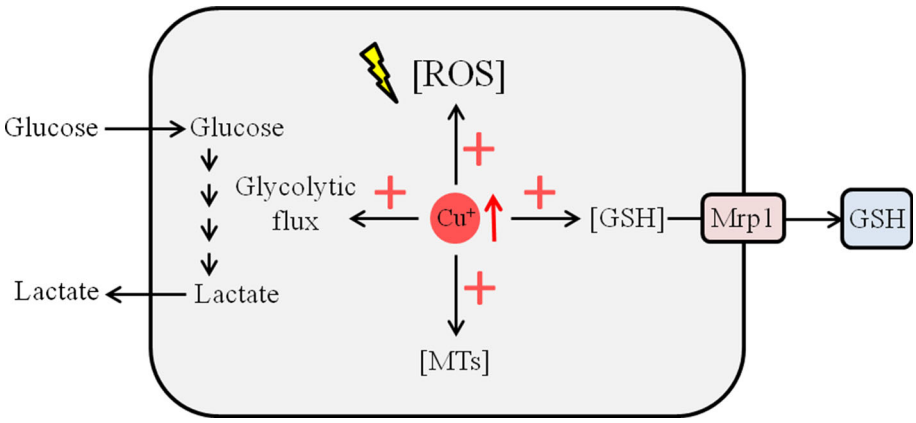

Fig. 3 Alterations in astrocytic metabolism after exposure to copper salts or CuO-NPs. Increased cellular copper levels strongly influence the metabolism of cultured astrocytes as indicated by increased contents of metallothioneins (MTs) and glutathione (GSH), an accelerated Mrp1-mediated GSH export and accelerated glycolytic glucose consumption and lactate production. In addition, elevated cellular copper levels can lead to an enhanced generation of reactive oxygen species (ROS)

suggesting that synthesis of a so far unknown protein is involved in the copper-induced stimulation of astrocytic glycolysis. Additionally, it was shown that the stimulation of the glycolytic flux is maintained even after the removal of extracellular copper [29, 33].

Treatment of cultured astrocytes with subtoxic concentrations of $CuCl_2$ [28] or copper oxide nanoparticles [33] strongly increased the cellular GSH content. As copper is quickly liberated from internalised CuO-NPs [96], it can be assumed that the elevated GSH content of astrocytes is also caused by the presence of an increased cellular concentration of copper ions. Elevated cellular copper concentrations may stimulate GSH synthesis by oxidative activation of γ-glutamate cysteine ligase [115], the enzyme limiting the rate of GSH synthesis. Alternatively, an increased uptake of the substrates cysteine or cystine, which are required for GSH synthesis in astrocytes [116], may accelerate GSH synthesis, thereby increasing the specific GSH content of astrocytes. Such an increase in cellular GSH content has also been reported for astrocytes treated with heavy metals and other compounds [16]. Currently, the molecular mechanism responsible for the observed elevated GSH content in astrocytes after exposure to copper ions or copper oxide nanoparticles remains to be elucidated. However, it appears to be at least independent of protein synthesis, since the increase in GSH content was not prevented by inhibition of protein synthesis (data not shown).

Inhibition of GSH export in astrocytes has also been reported to increase astrocytic GSH content [117, 118]. This GSH export is predominately mediated by the multidrug resistance protein 1 (Mrp1) [117, 119]. However, increased cellular GSH levels by inhibited basal Mrp1-mediated GSH export can be excluded as reason for the copper-induced elevated cellular GSH content, as the GSH export from such cells is actually accelerated [28, 33]. This elevated GSH export from astrocytes is mediated by Mrp1 [28] and is most likely a direct consequence of the elevated cellular GSH concentration in copper-treated astrocytes. As the K_M-value for GSH export from astrocytes is around 25 mM [120], an increase of the cytosolic GSH concentration from a basal value of 8 mM [101] by around 50 % [28, 33] will directly result in an accelerated Mrp1-mediated GSH export.

Conclusions and Perspectives

Cultured astrocytes have a higher tolerance against copper toxicity than neurons [80] and have the ability to efficiently take up, store and export copper, suggesting that also astrocytes in brain may have an important role in copper homeostasis. However, as the copper metabolism of other types of brain cells has not been investigated in detail, the potential role of other glial cells types and neurons in brain copper homeostasis remains to be elucidated. The ability of astrocytes to export copper suggests that these cells may supply copper to neighboring cells. However, the proposed copper supply function of astrocytes needs to be experimentally addressed and remains to be confirmed.

Copper has been discussed to plays a crucial role in the synaptic neurotransmission as modulator of neurotransmitter receptors [3, 121]. As astrocytes are an essential component of synapses and have the potential to efficiently take up and release copper, these cells may modulate synaptic copper levels. Such processes could be involved in the reported contributions of astrocytes to synaptic modulation and plasticity [8–12].

The emerging role of nanotechnology and the frequent application of nanomaterials increases the likelihood of an exposure of brain cells to nanoparticles. Astrocytes in culture deal well with various types of metal-containing

nanoparticles [78, 84] and tolerate also an exposure to moderate concentrations of CuO-NPs [24, 33], supporting the proposed role of astrocytes as a sink for potentially toxic metals [102, 122]. However, as soon as a threshold level of around 10 nmol copper/mg protein is exceeded, accelerated copper-mediated ROS production cannot be compensated anymore by the efficient astrocytic antioxidative defence system and cell toxicity is observed.

CuO-NPs are efficiently taken up into cultured astrocytes. This internalisation occurs most likely via endocytosis which directs the particles to the lysosomal compartment where copper ions are liberated to enter the cytosolic copper pool. As exposure of astrocytes to subtoxic concentrations of CuO-NPs has the same consequences as a treatment with copper ions (Table 2), including metabolic alterations such as increased contents of GSH and MTs and accelerated glycolytic flux, the observed consequences of an exposure of astrocytes to CuO-NPs are most likely a direct consequence of an increased cytosolic copper concentration. Nevertheless, the molecular mechanisms involved in CuO-NP internalisation, the vesicular trafficking and the potential lysosomal degradation of the particles remain to be elucidated.

Most of the data obtained on the handling of copper and CuO-NPs by astrocytes have been obtained on cultured primary astrocytes. Results of such studies have now to be verified for the in vivo situation to demonstrate for the brain the important function of astrocytes in copper homeostasis. In vivo experiments have shown that peripheral application of CuO-NPs result in cognitive impairments of rats [52]. In this context, it is important to investigate by which pathways applied CuO-NPs can reach in substantial amounts the brain and how such particles can compromise essential functions of the different types of brain cells.

Several neurological disorders, including Alzheimer's disease, Parkinson's disease, Wilson's disease and Menkes disease have been linked to an imbalance in the copper metabolism of the brain [3, 11, 114, 123, 124], suggesting that disturbances of astrocytic copper handling may be involved in the pathogenesis of such disorders. If this is the case, astrocytes would be an important target for therapeutic approaches to treat disorders connected with disturbances in brain copper metabolism.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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