

## HOMEOSTASIS OF ENERGY CONDUCTION, NEUROTRANSMITTERS, CYTOTOXIC COMPOUNDS AND METAL IONS

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Communication among unconnected cells requires the release of extracellular messengers and specific receptor mechanisms on, or in, the target cells. Signalling substances include hormones, neurotransmitter substances, trophic factors and diffusible substances. In higher organisms, synaptic transmission is the principal method of communication between cells, especially in the nervous system. Nerve cells mediate fast signalling between sensory systems and the central nervous system (CNS) and between the CNS and effector systems. Within the CNS, nerve cells form complex circuits that serve the integration of inputs and the generation of specific activity patterns. Synaptic transmission is the most important means by which nerve cells communicate. There are two principal types of synaptic transmission; electrical and chemical. Electrical transmission involves ion fluxes across membranes. In many cases, synaptic transmission is chemical and involves the secretion of signalling substances. In most cases, the termination of chemical transmission is achieved by rapid uptake of the released neurotransmitter by specific high-affinity neurotransmitter transporters into the synaptic terminal or the surrounding glial cells (Kuhar, 1973; Iversen and Kelly, 1975; Kanner, 1983, 1989; Kanner and Schuldiner, 1987). It has been known for many years that neurones and glia can accumulate neurotransmitters by Na<sup>+</sup>-dependent transport processes. Neurotransmitters are cotransported with Na<sup>+</sup> utilizing the energy stored in transmembrane electrochemical gradients generated by primary ion pumps (Kanner, 1983). Studies on neurotransmitter uptake have demonstrated the existence of multiple uptake systems, each relatively selective for a specific neurotransmitter. Neurotransmitters are transported across membranes by at least four distinct families of transporters: (1) vesicular transporters that function in the uptake of neurotransmitters into synaptic vesicles and granules (Schuldiner, 1994); (2) Na<sup>+</sup>- and Cl<sup>-</sup>-dependent (Na<sup>+</sup>/Cl<sup>-</sup>) transporters that operate on the plasma membrane of neuronal and glia cells (Uhl, 1992; Schloss *et al.* 1992; Amara and Kuhar, 1993); (3) Na<sup>+</sup>/K<sup>+</sup>-dependent transporters that function on the plasma membranes, especially in glutamate transport (Kanner, 1993); and (4) general amino acid transport systems that participate in controlling the availability of neurotransmitters outside the cells (McGivan and Pastor-Anglada, 1994).

Neurotransmission is very dynamic process that superficially appears to contradict homeostasis. However,

close examination of this process indicates the necessity of neurotransmitter homeostasis for brain function. The fate of monoamines and glutamic acid within and outside nerve cells is a good example of the dynamics of such homeostasis. Although glutamic acid can be accumulated in the cytoplasm of neurones to relatively high concentrations (millimolar) without apparent damage, similar concentrations outside the cell are cytotoxic and frequently lead to cell death (Attwell *et al.* 1993). Fig. 1 depicts some elements involved in neurotransmitter and metal-ion homeostasis in nerve cells. ATP is usually maintained at a high concentration in the cytoplasm and at a low concentration outside the cell. Mitochondria are the main providers of ATP except under oxygen stress. Most animals cannot survive prolonged anoxia without brain damage. Those animals that can survive prolonged anoxia utilize two main strategies to maintain high levels of ATP under oxygen deprivation: a drastic decrease in metabolic processes or an increase in their glycolytic activity (Perez-Pinzon *et al.* 1992; Lutz *et al.* 1995). Neuromodulators and neurotransmitters play a key role in the processes that are accompanied by changes in metal-ion homeostasis. ATP drives several primary pumps that function in the maintenance of the appropriate concentrations of neurotransmitters and metal ions in the various cell compartments. However, most of the energy for the transport systems is provided by two primary pumps: the Na<sup>+</sup>/K<sup>+</sup>-ATPase on the plasma membrane and the vacuolar H<sup>+</sup>-ATPase (V-ATPase) in the vacuolar system (Nelson, 1992). Consequently, most of the transport processes in the vacuolar system are driven by an electrochemical gradient of protons and most of the transport systems in the plasma membrane are driven by an electrochemical gradient of Na<sup>+</sup>.

Monoamines function as hormones and neurotransmitters in a variety of systems both within and outside the CNS. They are very reactive compounds and are cytotoxic at quite moderate concentrations (Schuldiner, 1994). Therefore, their homeostasis inside and outside the cell as well as their accumulation at high concentrations in specialized granules is one of the consequences of their cytotoxicity. Two principal transporters function in their homeostasis. One is driven by a Na<sup>+</sup> gradient and is located in the plasma membrane, and the other (vesicular monoamine transporter) is located in the vacuolar system and is driven by an H<sup>+</sup> gradient. Transporters similar to the mammalian vesicular monoamine transporters are present in bacteria. Remarkably, these transporters function

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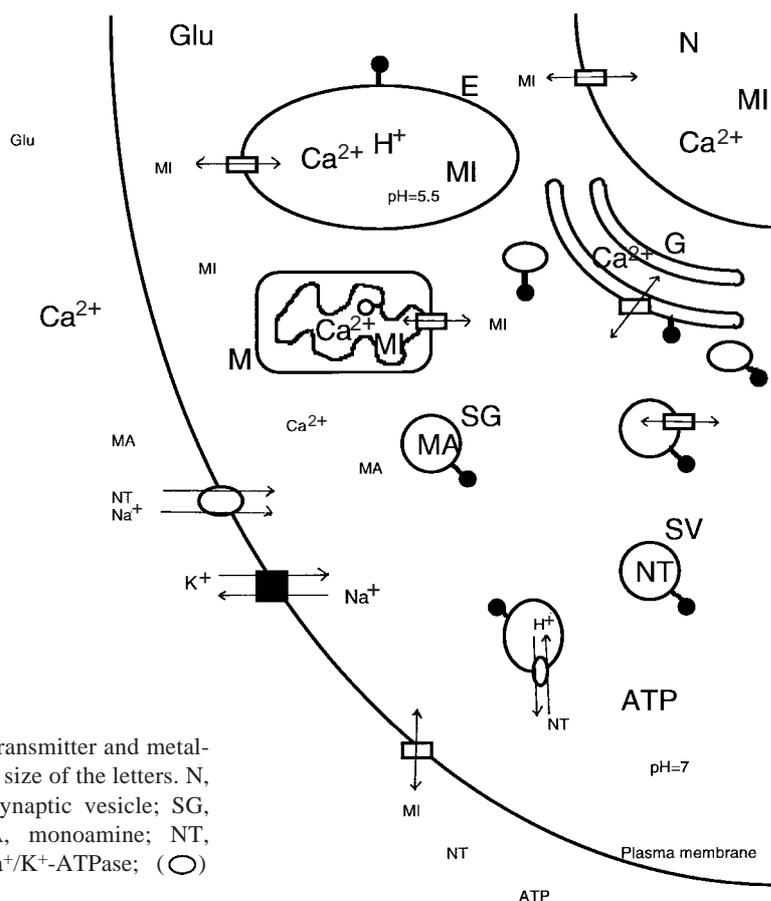


Fig. 1. Schematic depiction of the factors involved in neurotransmitter and metal-ion homeostasis. Relative concentrations are indicated by the size of the letters. N, nucleus; G, Golgi; M, mitochondria; E, endosome; SV, synaptic vesicle; SG, synaptic granule; Glu, glutamate; MI, metal ion; MA, monoamine; NT, neurotransmitter. (●) V-ATPase; (○) F-ATPase; (■) Na<sup>+</sup>/K<sup>+</sup>-ATPase; (○) neurotransmitter transporter; (□) metal-ion transporter.

in detoxification of the bacteria and can render them drug-resistant (Schuldiner, 1994). Evolution has apparently led to the utilization of pre-existing bacterial multidrug transporters for monoamine neurotransmission in the vertebrate brain.

The environment of unicellular and higher organism is laced with poisonous compounds, some of which are self-produced by their own metabolic pathways. To sustain life, the cells must get rid of these cytotoxic compounds and, in essence, achieve a homeostasis of the poison. The process of waste and poison removal from the cells involves special transporters that recognize the damaging compounds and transport them out of the cell. These transporters are divided into two main families, one utilizing the energy of ATP and the other utilizing the energy of ion gradients (Kanner, 1989; Nelson, 1992). Most transporters of both families have a very broad specificity for substrates and most of them are hydrophobic compounds, but they have no apparent structural similarity. Recent studies on the genes and cDNAs encoding these transporters shed some light on their general structure and function but have revealed no clues to the molecular mechanism mediating substrate recognition and translocation.

In contrast to monoamines, the homeostasis of glutamate in the brain is not influenced by the reactivity of glutamate but by the interaction between glutamate and specific receptors on the cell surface. Because glutamate is not a reactive compound, it can be accumulated to quite high concentrations in the

cytoplasm (see Fig. 1). Therefore, there is no need for a very active vesicular glutamate transporter, and the concentration of glutamate inside the synaptic vesicles may be only 10 times that in the cytoplasm (monoamines are present at a concentration more than four orders of magnitude higher than that in the cytoplasm). However, effective glutamate transport by the cytoplasmic membrane of nervous cells is crucial for the function and viability of these cells (Attwell *et al.* 1993). During hypoxia or ischaemia, glutamate transporters can run backwards, and the glutamate released reacts with its receptors, resulting in increased Ca<sup>2+</sup> entry into the cells. The high Ca<sup>2+</sup> concentrations inside the cells may trigger the death of neurones and thus cause brain damage.

Metal-ion homeostasis encompasses all the factors mentioned for neurotransmitters and poisonous compounds. Although metal-ion homeostasis is vital for every eukaryotic cell, it may serve a special function in certain organs and cell types. The presence of unusual concentrations of metal ions can cause impaired brain function or cell death. The different ions may be distinct as redox-active ions such as Fe<sup>2+</sup>, Cu<sup>2+</sup>, Co<sup>2+</sup> and to a lesser extent Mn<sup>2+</sup> or non-redox-active ions such as Ca<sup>2+</sup> and Zn<sup>2+</sup>. Zn<sup>2+</sup> and Ca<sup>2+</sup> may be targeted to transcription factors and other enzymes involved in DNA metabolism. Targeting redox-active metal ions to these places can lead to the promotion of radical reactions that result in nucleic acid damage. The redox-active ions normally function

in enzymes that participate in redox reactions and in the conversion of active oxygen-containing components. All of these processes require defined amounts of specific metal ions at the right position and at the right time. In brain cells,  $Zn^{2+}$  is accumulated in presynaptic vesicles of excitatory neurones and is released during synaptic activity (Assaf and Chung, 1984; Howell *et al.* 1984).  $Zn^{2+}$  interacts with some ionotropic receptors in the brain. For example, the ionotropic ATP receptor (P2x3) is potentiated by  $Zn^{2+}$  (Seguela *et al.* 1996) and it blocks currents mediated by *N*-methyl-D-aspartate (NMDA) or  $\gamma$ -aminobutyric acid (GABA) as well as voltage-gated  $Ca^{2+}$  channels (Westbrook and Mayer, 1987; Peters *et al.* 1987). Since  $Zn^{2+}$  is secreted from synaptic vesicles, interacts with certain receptors and should have a specific transport system, it may be considered as a neurotransmitter. All of these considerations call attention to the importance of metal-ion homeostasis in brain cells. Through a mutation in the yeast gene *CDC1*, a gene encoding a manganese transporter (SMF1) has been identified (Supek *et al.* 1996). It was observed that the yeast *SMF1* gene shares homology with the mouse *Nramp* gene. *Nramp* (*Bcg*) was cloned as a gene responsible for mouse resistance to infection with mycobacteria and is identical with the *Ity* and the *Lsh* genes conferring resistance to infection by *Salmonella typhimurium* and *Leishmania donovani*, respectively. We propose that the mammalian protein, like the yeast transporter, is a  $Mn^{2+}$  and/or  $Zn^{2+}$  transporter. This result led to the proposal that *Nramp* functions in the induction of resistance or sensitivity to mycobacteria (Supek *et al.* 1996). The hypothesis is based on manganese homeostasis in macrophage phagosomes that are maintained at low concentration to reduce the mycobacteria infectivity.

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