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Amino Acids

Transport proteins: targets for glutamate pharmacology?

M. Herrera-Marschitz

Editorial

Amino Acids, the building blocks of proteins, are still in the focus of the journal, even if with the advent of proteomic methods is now possible to investigate in almost one step a large number of protein phenotypes (Fountoulakis, 2001). Thus, by example, the proteomic analysis of a single lumbar puncture allowed to characterise more than 50 different gene products simultaneously in the cerebrospinal fluid of patients with schizophrenia. However, the only difference that was found was in apolipoprotein A-IV levels (Jiang et al., 2003), a protein associated with cholesterol transport (Steinmetz et al., 1990).

While aware that the uniqueness of an individual is due almost entirely to the type of proteins it produces, not all-possible proteins are expressed at any time point. Furthermore, a single protein may be involved in more than one process, and similar functions may be carried out by different proteins or by their products, peptides and simple amino acids. Therefore, neurobiology is still focusing on single amino acids, in particular on those proposed as neurotransmitters, attempting to understand how specific messengers are synthesised, stored in nerve terminals, released upon an action potential or other mechanisms, and bind to receptor-proteins.

Glutamate is still a very attractive molecule, a nonessential amino acid playing a fundamental role in the synthesis of proteins, but also acting as a major excitatory neurotransmitter in the mammalian CNS (Watkins and Evans, 1981; Fonnum, 1984). Glutamate is present at $\sim 10\,\mu\mathrm{mol/g}$ in brain tissue, and found at $1\,\mu\mathrm{M}$ concentration in the extracellular space of different brain regions of the rat (see Herrera-Marschitz et al., 1992, 1997, 1998), where glutamate is released upon depolarisation in a α-latrotoxin-dependent manner (Herrera-Marschitz et al., 1996). Glutamate is fundamental for development and neuronal plasticity, is involved in several modulating functions, but can also produce cellular damage (see McDonald and Johnston, 1990). Glutamate has been linked to several diseases and, indeed, following metabolic disturbances glutamate overflow is increased, resulting in (i) glutamate receptor over-stimulation, (ii) sustained depolarisation, (iii) metabolic imbalance and (iv) neuronal damage (see Schmidt, 1998; Schmidt and Herrera-Marschitz, 1998; Herrera-Marschitz and Schmidt, 2000; Kretschmer et al., 2002).

Olney (1969) first demonstrated that L-glutamate could be highly toxic to the brain, proposing the term excitotoxicity. Hence, glutamate has been used as an experimental neurotoxin and can induce convulsions in rats upon systemic administration (Peñafiel et al., 1991). The term excitotoxicity is now widely applied to the effects produced by endogenous and exogenous substances via glutamate receptors (see Novelli and Tasker, 2002). Excitotoxicity plays a role in neurodegeneration (see Kostrzewa 1998; Sonsalla et al., 1998) and it has been proposed that glutamate receptor antagonists are promising drugs to prevent neurotoxicity (see Danysz et al., 1998; Parsons et al., 1998; Tzschentke, 2002).

Overflow of glutamate is regulated by a potent transport system, to neurons and to astrocytes, and by a complex and sophisticated set of receptors, whose number and functions are still largely unexplored (see Kretschmer et al., 2002).

A complex family of Na^+/K^+-dependent glutamate transporters has been characterised (Amara, 1992), comprising five different isoforms, EAAT1 to 5 (see Mason et al., 1999), with rather different channel properties, and cellular and regional distributions. In the framework of the 6$^{\text{th}}$ International Congress on Amino Acids in Bonn, 1999, Danbolt and co-workers anticipated an explosive increase in the number of studies on glutamate clearance

by multiple transport proteins (Lehre and Danbolt, 1999; Gegelashvili et al., 1999; Plachez et al., 1999).

EAAT1 (GLAST) and EAAT2 (GLT-1) are expressed by astrocytes, exclusively in the brain, with EAAT2 particularly expressed in the basal ganglia (Anderson and Swanson, 2000). These two transporters are fundamental for glutamate clearance, since down regulation leads to a dramatic increase in extracellular glutamate levels and excitotoxicity (Rothstein et al., 1996). The EAAT3 (EAAC1) and EAAT-4 are mainly expressed by γ-aminobutyric acid (GABA) neurons, probably to store enough glutamate to then via Glutamic Acid Decarboxylase (GAD) be decarboxylated to GABA (Rauen, 2000). Interestingly, however, down regulation of EAAT3/4 does not lead to an increase in extracellular glutamate levels. EAAT1/2 are regulated by glutamate and cAMP analogs, while EAAT3 is regulated by PKC phosphorylation (see Masson et al., 1999). The expression of EAAT5 is stronger in retina than in any other tissue and, like that for EAAT4, is associated to Cl^- conductance, suggesting that these two proteins may rather function as modulators than as transporters (Rauen, 2000). There is evidence indicating that glutamate transporters are vulnerable to the action of biological oxidants, resulting in reduced uptake function and accumulation of extracellular levels of glutamate. A series of redox sensing elements consisting of cysteine residues have been identified in the structures of EAAT1, EAAT2 and EAAT3 (see Trotti et al., 1998), suggesting that antioxidant agents may improve glutamate transport diminishing the deleterious effect produced by enhanced glutamate stimulation. This is indeed an area deserving further investigation, promising to reveal new targets for pharmacological interventions.

Upon synthesis in nerve terminals, glutamate has to be loaded into synaptic vesicles until released in response to an action potential. As for several classical neurotransmitters, there is evidence for glutamate vesicular transport (Disbrow et al., 1982; Shioi et al., 1989; Tabb et al., 1992), and two members of the Na^+-dependent inorganic phosphate transporter family have been established as vesicular glutamate transporters, i.e. VGLUT1 and VGLUT2 (see Gras et al., 2002). Both transporters are abundantly expressed in the brain (Ni et al., 1994, 1995; Hisano et al., 1997, 2000), and found in vesicles located in terminals forming asymmetric contacts (Bellocchio et al., 1998; Fremeau et al., 2001; Varoqui et al., 2002). VGLUT1 is present in excitatory neurons from cerebral and cerebellar cortices and hippocampus, and VGLUT2 in neurons from diencephalon and rhombencephalon (Fremeau et al., 2001; Varoqui et al., 2002).

Recently, Gras et al. (2002) reported above a third vesicular glutamate transporter (VGLUT3), expressed by acetylcholine (ACh) interneurons of the neostriatum and 5-hydroxytryptamine (5-HT) neurons of the raphé nuclei. The finding is exciting, and fully possible from the requirement of local synthesis, because glutamate is produced everywhere in the mitochondria, by transamination of α-ketoglutarate. The finding that VGLUT3 co-localises with the vesicular ACh transporter (VAChT) in the neostriatum is also exciting because, upon depolarisation, the large striatal interneurons could simultaneously release both ACh and glutamate. Indeed, in a microdialysis study it was shown that the selective cholinergic neurotoxin AF64A administered into the neostriatum produced a simultaneous decrease in K^+-evoked ACh and glutamate release (Meana et al., 1992).

Using an antiserum raised against aspartate conjugated to keyhole-limpet hemocyanin, aspartate-positive neurons were demonstrated in the neostriatum of the rat, modulated by the D1-dopamine subtype (Pettersson et al., 1996; Herrera-Marschitz et al., 1997). The co-localisation of ACh and aspartate markers was not investigated in that study, although the morphological features of the aspartate-positive neurons were similar to smaller aspiny, rather than to larger cholinergic interneurons. Nevertheless, the possibility of a co-localization of aspartate and/or glutamate and ACh markers has to be further investigated.

The main source of glutamate for neurotransmission is that produced by the glutamine cycle, involving glutamine synthetase and glutaminase, which are selectively located in astrocytes and glutamate- and GABA-containing nerve terminals, respectively (Ottersen and Storm-Mathisen, 1987; Laake et al., 1995). The glial glutamine produced by glutamate via glutamine synthetase is transferred to the neurons involving a specific transport system (Broer et al., 2001). The removal of glutamine from glial cells appears to be achieved by a protein named SN1, a System N transporter family member located on astrocytes (Chaudhry et al., 1999), while the transfer to the neurons appears to be achieved by a protein named SAT1/AT1, a System A family member located on neurons (Varoqui et al., 2000; Weiss et al., 2003). Whether SN1 and/or SAT1/AT1 glutamine transporter systems will provide a target for drugs acting on amino acid containing neuronal systems remains to be investigated.

In conclusion, amino acids are still in the focus of exciting research, because apart from being the building blocks of proteins; they play a fundamental role as neurotransmitters, involved in development and neuronal plasticity. Glutamate constitutes the main excitatory neurotransmitter,

which upon metabolic disturbances may be transformed into a neurotoxin, and be associated with several neurodegenerative disorders. For preventing excessive glutamate accumulation in the extracellular space there is a potent carrier system mainly to glial cells shaping the homeostasis of the amino acids. The identification and functional evaluation of each protein involved in the transport of amino acids may constitute a goal for developing new drugs aiming glutamate systems in the CNS.

Thus, the Editors of *Amino Acids* foresee 2004 as an exciting year for amino acid research revealing new mechanisms, new understandings on the role of amino acids in several disease states and new opportunities for drugs targeting specifically amino acid neurotransmission. We wish that *Amino Acids* is established as the pioneering forum for protein, peptide and amino acid research, constituting the first choice for publishing original manuscripts and opinions leading to new developments in the field, also in Neurobiology and Medicine.

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Authors' address: Prof. Mario Herrera-Marschitz, Programme of Molecular & Clinical Pharmacology, ICBM, Medical Faculty, University of Chile, Santiago, Chile, E-mail: mh-marschitz@med.uchile.cl; Dept. of Physiology & Pharmacology, Karolinska Institutet, Stockholm, Sweden, E-mail: Mario.Herrera-Marchitz@fyfa.ki.se