Review

Molecular Pharmacology of the Na⁺-Dependent Transport of Acidic Amino Acids in the Mammalian Central Nervous System

Vladimir Josef BALCAR¹⁾

Department of Molecular Pharmacology, Faculty of Pharmaceutical Sciences, Kanazawa University,
13-1 Takara-machi, Kanazawa, Ishikawa 920-0934, Japan. Received December 17, 2001

The Na⁺-dependent transport of L-glutamate (GluT) has been identified in brain tissue more than thirty years ago. Neurochemical studies, performed in various experimental models during 1970's, defined the basic rules for the selection or synthesis of GluT-specific substrates and inhibitors. The protein molecules (transporters) that mediate the translocation of the substrates across the plasma membrane have been cloned and studied during the last ten years. The sites on the transporters that bind the substrates favour glutamate-like or aspartate-like molecules with one positively charged and two negatively charged ionised groups. Substituents at C3 and C4 are often tolerated but substitutions at C2 or alterations of the ionisable groups usually impede the binding. The substrate binding sites display an "anomalous" selectivity towards stereoisomers. These structural requirements are shared by all Na⁺-dependent glutamate transporters thus making the design of transporter-selective ligands a challenging task. Moreover, the molecular mechanisms of the transport have not yet been adequately elucidated. Data from a wide variety of experimental studies strongly indicate that Na⁺-dependent GluT regulates the functioning of the glutamatergic excitatory synapses—the most important rapid inter-neuronal signalling system in the mammalian brain. Altered structural and/or functional properties of the Na⁺-dependent glutamate transporters have been implicated in the damage to the brain tissue following cerebral ischaemia and in the progressive loss of neurons in conditions such as Alzheimer dementia and amyotrophic lateral sclerosis. Furthermore, it seems that fine-tuning of glutamatergic neurotransmission by regulating the Na⁺-dependent GluT could be useful in the therapy of schizophrenia.

Key words glutamate; neurodegeneration; brain ischaemia; neurotoxicity; aspartate; neuroleptic

HISTORICAL OVERVIEW

The crucial discovery of the excitatory properties of acidic amino acids^{2–5)} lead only gradually and with considerable delay, to the formulation of the hypothesis of the synaptic role of L-glutamate in the mammalian central nervous system (CNS)^{6–9)} (for reviews of early history of amino acid research, see^{10–13)}). It may never be possible to identify any single experimental study after which L-glutamate became unequivocally accepted as the most important excitatory synaptic transmitter in the brain and spinal cord, but, it is certain that the discovery of the "high affinity uptake" accumulating L-glutamate into "synaptosomes"^{14,15)} (for reviews see^{13,16,17)}) was an important step in the development of the concept of the glutamatergic neurotransmission in the CNS. As a putative mechanism for the necessary removal and inactivation of extracellular L-glutamate it provided a target for pharmacological studies and helped to establish a neurochemical link between L-glutamate and the mechanisms of synaptic transmission.^{13,18,19)} Most of the latter experiments were carried out *in vitro* using tissue "mini-slices" prepared from the rat cerebral cortex or cat spinal cord (for details of the methodology see^{20,21)}). Rather than disrupting the nervous tissue by homogenization, the technique was using what might be called "neurochemical dissection". In more specific terms, it examined a large number of compounds, mostly glutamate and aspartate analogues but also a range of drugs, for possible effects on L-glutamate transport (GluT). Such approach had a potential to yield valuable information on the molecular properties of the substrate-recognition site on GluT,¹⁹⁾ but, also, by testing neuroactive compounds with known sites of actions in brain, it helped to formulate hypotheses defining the most probable functions of the GluT in

the mechanisms of the excitatory synaptic transmission.

The pharmacology and biochemistry of GluT was later extensively investigated in cultured glial cells^{22–24)} (for a review see²⁵⁾) but it was the data obtained in anaesthetised animals, using compounds (D- and L-threo-3-hydroxyaspartate, DL-*t*-3OHA) identified earlier as powerful specific inhibitors of [³H]L-glutamate uptake in brain slices,²⁶⁾ that provided the first convincing evidence that GluT could influence the excitatory actions of L-glutamate *in vivo*, at least in the cat spinal cord.²⁷⁾ Furthermore, if the inhibition of GluT *in vivo* caused an increase in the extracellular concentrations of L-glutamate then, given the neurotoxic nature of excessive glutamatergic excitation,^{28,29)} similar inhibition of GluT applied to functioning synapses should, under suitable experimental conditions, result in the appearance of neurodegeneration. Such experiment was indeed performed and it was shown that an injection of DL-*t*-3OHA into the rat striatum could cause the death of brain cells *in vivo*.³⁰⁾

HETEROGENEITY OF GLUTAMATE TRANSPORT

During the 1980's and early 1990's, it became apparent that, in order to account for the variations of GluT characteristics from one experimental model to another, there had to be several distinct transport systems contributing to the Na⁺-dependent uptake of L-glutamate into brain cells.^{31–34)} The evidence was obtained using both cultured cells and rat brain tissue (for a review see³⁵⁾). Strong evidence of differences between GluT in, respectively, glial cells and neurons emerged³⁴⁾ but variations in GluT characteristics among brain regions, particularly between the forebrain and the cerebellum, were also reported.^{31,32)} Eventually, several distinct transporters were cloned and characterised^{36–42)}, for re-

* To whom correspondence should be addressed. e-mail: vibar@anatomy.usyd.edu.au

cent reviews and additional references see.^{13,16,17,43–46)}

Glutamate transporters cloned initially from rat brain tissue or rabbit gastrointestinal tract were named in a somewhat haphazard manner (GLutamate Transporter, GLT; GLutamate and ASpartate Transporter, GLAST; Excitatory Amino Acid Carrier, EAAC). The designations EAAT1 (Excitatory Amino Acid Transporter 1), EAAT2, EAAT3, *etc.*, follow the systematic nomenclature preferentially used when referring to the transporters cloned from human brain tissue but it should be kept in mind that the glutamate transporters (EAAT's) have now been cloned in several laboratories, from several species and, in addition, a number of splice variants have been identified so that the nomenclature has become somewhat less simple than that shown in Table 1 (for reviews see^{16,17,43–46)}).

In the remainder of the text I shall use the abbreviation EAAT when referring to a glutamate transporter in a generic manner or to all glutamate transporters as a group. In all other cases I shall prefer the original names given to the transporters when they were cloned, *i.e.* GLAST, GLT, EAAC, EAAT4 and EAAT5 (Table 1). When this could give rise to confusion in relation to an original reference I shall use the specific EAAT designation in parentheses, *e.g.* GLT (EAAT2). The abbreviation GluT is used when referring to the Na⁺-dependent L-glutamate transport in general, *i.e.* not to any individual transporters.

LOCATION OF GLUTAMATE TRANSPORTERS IN THE CNS

Cloning and sequencing of transporter proteins has made it possible to prepare antibodies against individual EAAT's and to study their location in brain regions and brain cells using immunocytochemistry^{47,48)} (see^{16,17)} for additional discussion and references). The technique has been extensively exploited in a variety of studies^{47,49–54)}; for instance, it has been used to quantify the abundance of EAAT proteins in terms of numbers of molecules per volume of tissue (Fig. 1; *cf.* ref.^{52,53)}; see¹⁶⁾ for a review). Earlier, there had been numerous attempts to study regional distribution of GluT by autoradiographical visualisation of tritiated radioligands bound to GluT sites, either *in vivo* (for reviews see^{8,17)}), or, on sections of frozen rat brain *in vitro*.^{55,56)} The autoradiographic studies based on the labelling of brain sections *in vitro* will be discussed in more detail later because of their potential to show not only the regional distribution of the substrate-binding sites on GluT but, at the same time, also their neurochemical and pharmacological characteristics. The preferred radioligands in those experiments were either [³H]D-aspartate^{57–63)} or [³H]L-aspartate.^{62–67)} Finally, *in situ* hybridisation (ISH) was employed to study regional and cellular distribution of GluT.^{49,68–70)}

In some cases, the results of ISH studies are not in complete agreement with the immunocytochemistry. While immunocytochemistry has indicated that GLT is present only in astrocytes, at least in the adult healthy mammalian (rat) brain, a GLT mRNA has been observed also in neurons, in addition to glial cells.^{49,68–70)} Either the neurons contain an as yet unidentified GLT splice variant that is not recognized by any of the antibodies so far made against various peptide sequences of the GLT molecule or, the level of expression of

GLT in neurons is too low for the sensitivity of immunocytochemical methods to detect it.^{16,17)} Either way, it demonstrates limitations of experimental investigations relying solely on the use of antibodies (for more detailed criticism, see ref.¹⁷⁾), or, on the use of just one technique, in general (see also the final section of this article).

The methodological caveats mentioned in the previous paragraph are important, because much of the information on the distribution of the individual transporter molecules, as displayed in Table 1, has been obtained by immunocytochemistry or by Western blotting, *i.e.* again by using EAAT-specific antibodies (for reviews see^{16,17)}). GLT is usually said to be most abundant in the forebrain, particularly in the hippocampus, but it has also been found in the cerebellar cortex, in the brain stem and in the spinal cord. In all regions of the normal adult brain so far studied, GLT immunoreactivity has only been detected in glial cells. GLAST has also been found in all parts of brain⁴⁷⁾ (for reviews see^{16,17)}). While GLAST has been reported to exist in astrocytic membranes alongside of GLT,⁷¹⁾ data from cultured cells suggest that GLT and GLAST may exist in separate, morphologically distinct types of astrocytes.⁷²⁾ A recent electrophysiological study performed in freshly isolated glial cells has indicated that there are indeed two distinct types of astrocytes in the hippocampus that differ in their functional expression of GluT.⁷³⁾

While GLT seems to be the principal glutamate transporter in the forebrain glial cells, GLAST is by far the most abundant glutamate transporter in the cerebellar cortex (at least on the basis of "quantitative" immunocytochemistry,¹⁶⁾ *cf.* Fig. 1). The overall distribution of EAAC (EAAT3) is more difficult to assess (for reviews see^{16,17)}). The EAAC (EAAT3) transporter protein has been identified in many parts of the CNS and it has been sometimes presented as a typical "neuronal" glutamate transporter.⁷⁴⁾ This may, however, be an oversimplification, since EAAC (EAAT3) has also been found in non-neuronal cells such as oligodendrocytes and cultured glioma cells.^{75–77)} While both GLAST and GLT appear to be associated mostly with cytoplasmic membrane, EAAC (EAAT3) has been found predominantly inside the cells (for a review see¹⁶⁾). One other glutamate transporter that is found almost exclusively in neurons, specifically in the Purkinje cells of the cerebellar cortex, is EAAT4.⁴¹⁾

There are, however, at least two types of mammalian tissue where the distribution of EAAT's does not follow the pattern summarized in Table 1: immature brain cells, often studied in culture, and the retina. In the cultured cells, GLT has been

Table 1. Sodium-Dependent Glutamate Transporters of the Mammalian Central Nervous System

Transporter	Principal location in the adult CNS	References
EAAT1 (GLAST)	Bergman glia of the cerebellar cortex, Astrocytes throughout the CNS	16, 17, 48, 53
EAAT2 (GLT)	Astrocytes throughout the CNS, mRNA found in neurons	16, 17, 48, 53
EAAT3 (EAAC)	Neurons of the CNS	16, 17
EAAT4	Purkinje cells of the cerebellar cortex	16, 17
EAAT5	Retina	16, 17

Some of the transporters have been found to be expressed at low levels by certain non-neural tissues but this subject is not discussed in the present article. In many earlier publications the transporters were often labelled as "GLT-1" or "EAAC1" but this "numbering" has been abandoned in this review in accordance with more recent usage.^{16,17)}

Fig. 1. Average Densities of Sites Labelled by 20 nM [³H]L- and [³H]D-Aspartate Compared to the Distribution of GLAST (EAAT1) and GLT (EAAT2)

The binding of radioligands was analysed autoradiographically (for details of the method see^{63–67}). The distribution of the GLAST (EAAT1) and GLT (EAAT2) is based on the immunocytochemical studies of Danbolt *et al.* (see ref.^{16,48,53}). The radioligand binding (a, b) is expressed as fmol/mg protein, mean $\pm$ S.D. of 6–12 values; (c, d) are estimates of the numbers of EAAT molecules per μm^3 of tissue. NCx, neocortex; Hpp, hippocampus; Str, striatum; SpN, septal nuclei; CbCx, cerebellar cortex.

found not only in astrocytes but also in neurons, particularly in hippocampal cultures^{78–83}) and in Purkinje cells originating from an avian cerebellum.⁸⁴) In some cases the immunocytochemical detection has been supported by data on uptake^{84,85}) so that the evidence of a functional, GLT-mediated (or GLAST-mediated^{83,85}) glutamate transport located in such neurons is very convincing. The expression of GluT in developing neurons *in situ* also seems to take place but it appears to be a transient phenomenon,⁸⁶) perhaps typical of the regions of developing brain undergoing rapid cell proliferation and/or synaptogenesis.⁸⁷)

The distribution of glutamate transporters in the retina has recently been reviewed in great detail.^{88,89}) Immunocytochemistry has indicated neuronal location of GLT in the retina while GLAST seems to be present only in glial cells. Retina, however, presents more complex picture since it also contains EAAT5, a transporter that appears to have only very low levels of expression in other parts of the CNS.^{42,90}) The function and location of amino acid transporters in the retina presents a number of challenging issues that will not be specifically addressed here (see^{88,89}) for reviews).

SUBSTRATE SPECIFICITY AND STRUCTURAL REQUIREMENTS OF GluT

Early studies indicated that the most important requirement of the Na⁺-dependent transport of L-glutamate was that the substrates have one positively charged and two negatively charged ionic groups.¹⁹) This conclusion was made after testing many glutamate and aspartate analogues for possible inhibitions of [³H]L-glutamate uptake in slices of rat cerebral cortex.

The uptake was strongly stereoselective, favouring L- over D-glutamate, but this stereoselectivity did not extend towards shorter carbon skeletons such as aspartate where both D- and L-isomers interacted with the uptake.¹⁹) This characteristic was subsequently referred to as “stereoselective anomaly” by a group of biochemists.⁹¹) Several explanations for this particular structural requirement have been put forward.^{67,92}) Among other main features of the substrate-selectivity was an aspartate analogue with a substitution on C2 (α carbon atom, 2-methyl-DL-aspartate) having a lower affinity for uptake than either the aspartate enantiomers or L-glutamate, while, in contrast, substitutions on C3 were well tolerated at least in the case of aspartate analogues (DL-*t*3OHA). Large difference between *threo* and *erythro* isomers of DL-3OHA indicated that the optimum bound conformation was narrowly defined and any structural alterations that would prevent the substrate molecule from attaining it could result in a major loss of the ability to interact with the substrate-recognition site. More detailed study of *t*3OHA showed, however, only a very small difference between the D- and L-enantiomers in the affinity for GluT.²⁶) Another example of a significant difference in the affinities of two apparently very similar analogues is the Glu molecule with substitutions on C4: 4-fluoro-L-glutamate and 4-methylene-L-glutamate.¹⁹) While the 4-methylene substitution at C4 (Fig. 2) results in the affinity substantially lower than that of L-glutamate, a fluoro-substitution at the same carbon atom has no such effect (involvement of specific characteristics of the fluorine atom in the observed inhibition cannot be, however, ruled out).

Re-positioning amino group from C2 onto C3 did not change the affinity; 3-aminoglutarate (β -glutamate) had about the same IC₅₀ v. [³H]L-glutamate uptake as the non-ra-

Fig. 2. Structural Formulae of Some of the Substrates/Inhibitors of the Na^+ -Dependent Glutamate Transport in the CNS

Abbreviations: L-CCG III, (2S,1'S,2'R)-2-(carboxycyclopropyl)glycine^{102–104}; L-*a,e*-MPDC, L-*anti,endo*-3,4-methanopyrrolidinedicarboxylic acid^{109,110}; L-t-PDC, *trans*-pyrrolidine-2,4-dicarboxylate^{100,110}; DL-TBOA, DL-*threo*- β -benzyloxyaspartate.^{115,116}

diactive L-glutamate. However, extending the length of the carbon atom chain resulted in a rapid loss of activity, with 3-aminoadipate displaying only low affinity for the site and 2-aminoadipate being inactive. Replacement of carboxyl by sulphate group was well tolerated and the affinity remained high (L-cysteate, L-serine-*O*-sulphate) but any substitution leading to the loss of charges on the ionised groups rendered the resulting analogues inactive.¹⁹ It is, however, interesting to note that, when sulphate replaced carboxyl in an aspartate analogue, a strong stereoselectivity appeared (D-cysteate was inactive¹⁹). In a study done more than 25 years later, using a very different experimental model, many of the initial results were reproduced with only minor differences that could be explained by a greater precision of the more recent technique. The experimental model used in those studies was the uptake of [^3H]L-glutamate by cultured human fibroblasts that were shown to express at least four glutamate transporters⁹² and the most significant departure from the data in brain slices was that of β -glutamate appearing as a “weak” rather than “strong” inhibitor.

In the intervening years, however, several studies using either cultured cells or preparations derived from fresh brain tissue showed significant variations from the substrate selectivity described for [^3H]L-glutamate uptake in the slices of rat cerebral cortex, indicating possible existence of transport systems preferring L- over D-aspartate (cerebellar cortex³¹), strongly preferring L-glutamate over either L- or D-aspartate (cultured neuroblastoma cells⁹³) or D- and L-aspartate over L-glutamate.⁹⁴ In some cases, GluT was found to tolerate hydrazide- or hydroxylamine-analogues^{95,96}; for reviews

see.^{34,35} The general pattern of structural selectivity of GluT, as set by the data obtained in rat cerebral cortex slices^{19,26} was, however, confirmed in all major studies using synaptosomes or cultured cells.^{97,98}

In 1979, dihydrokainate (DHK) was shown to be a relatively specific, if not very potent, inhibitor of [^3H]L-glutamate uptake⁹⁹ and this pointed the way towards the preparation of similar, conformationally restricted structural analogues of glutamate or aspartate that would be more potent and could be used as highly specific probes for studies of GluT. Since the late 1980's, several such substrates or inhibitors of GluT have been synthesised (Fig. 2) and tested. L-*trans*-Pyrrolidine-2,4-dicarboxylate (L-tPDC,¹⁰⁰ Fig. 2) has become the most widely used pharmacological marker for GluT despite initial reports suggesting that it might not be as potent substrate or inhibitor of GluT as some of the earlier identified compounds.¹⁰¹ Another conformationally-restricted glutamate analogue (2S,1'S,2'R)-2-(carboxycyclopropyl)glycine (L-CCG III^{102,103}) is actually a naturally-occurring substance¹⁰⁴ (Fig. 2, for a review see¹³). It was found to have a very high affinity and specificity for GluT¹⁰⁵ (for a review see¹³) and recent studies using NMR analyses have shown that L-CCG III can be very rapidly accumulated by brain slices, even against steep concentration gradients.¹⁰⁶ Other conformationally-restricted glutamate analogues have been synthesised and used as substrates/inhibitors of GluT^{107–109}; for a review see.¹¹⁰ In some cases, such compounds have been shown to be transporter selective.¹¹¹ Among the latest additions to the armoury of GluT-specific pharmacological probes have been glutamate analogues without the ring structures restricting the movement of the main pharmacophores in their molecules. Rather, these recently introduced analogues contain specifically placed substituents. Thus some 3- and 4-methyl-substituted glutamate molecules have been shown to distinguish between GLAST (EAAT1) and GLT (EAAT2) in experiments using the transporters expressed in *Xenopus laevis* oocytes,¹¹² (2S,4R)-4-methylglutamate (Fig. 2) was identified as a very potent inhibitor of GluT in cultured hippocampal neurons¹¹³ and ($\pm$)*threo*-3-methylglutamate, one of the glutamate analogues identified earlier as being transporter-selective¹¹² in *Xenopus laevis* oocytes has now been evaluated for transporter-selectivity in cultured fibroblasts expressing GLAST (EAAT1), GLT (EAAT2) or EAAC (EAAT3).¹¹⁴ DL-*threo*- β -Benzyloxyaspartate (TBOA, Fig. 2), another one of such analogues,^{115,116} has turned out to be the most potent non-transportable inhibitor of GluT identified to date (but see also ref.¹¹⁷) and has already been proving useful in studies of the role of GluT at glutamatergic synapses, cf. e.g. ref.^{117–121}

ROLES OF GLUTAMATE TRANSPORT IN HEALTH AND DISEASE

Glutamatergic Synapses Association of the Na^+ -dependent high affinity uptake of L-glutamate with synaptosomal fractions^{14,15,122} prompted suggestions that GluT plays a specific role in the function of glutamatergic synaptic transmission (for a review see ref.¹³). Reuptake into synaptic terminals could remove and inactivate synaptically-released L-glutamate and this could be essential to keep the transmission of information at glutamatergic synapses sharp and

crisp.¹²³) Given the pivotal role of the glutamatergic transmission in the mammalian brain tissue, changes in the activity of GluT—whether it be decreases or increases—would be expected to have severe impact on the function of the CNS. For example, it was suggested that GluT could be linked to the loss of motor neurons in the amyotrophic lateral sclerosis (ALS); the neuronal death could be a consequence of extracellular L-glutamate reaching neurotoxic levels as a result of an inadequate removal by deficient GluT.¹²⁴) The role of GluT at glutamatergic synapses has not, however, been always unequivocally accepted (for a review *cf.* ref.¹²⁵) and the link between a change in GluT and the death of motor neurons in ALS is not easy to test.

Glutamate Neurotoxicity and Cerebral Ischaemia

Under certain conditions, L-glutamate was found to be released—or to “spill out”—from synaptosomes *in vitro*¹²⁶) and this may have been linked to the failure of GluT because of the lack of metabolic energy derived from glucose¹²⁷) (but see also¹²⁸). It has been further proposed that similar phenomena could occur in pathological states, such as ischaemia, when GluT would be actually reversed, pumping L-glutamate out of the cytoplasm back into the extracellular space.^{129–131}) As GluT is driven by ionic gradients, the “reversed” transport might result from the collapse of such gradients under anoxic/hypoglycaemic conditions as would be encountered in the acute cerebral ischaemia, a state that is characterized by high extracellular concentrations of K^+ . It has been argued, however, that an increase in the extracellular K^+ might not be enough to cause the complete reversal of GluT because of compensatory mechanisms¹³²) (for a discussion see¹³³). It has nevertheless been demonstrated that the glutamate release induced by experimental ischaemia *in vivo* is sensitive to the non-transportable, GLT-selective GluT inhibitor dihydrokainate thus suggesting that it could indeed be mediated by “reversed” GLT.¹³⁴) DHK has been shown to reduce the release of [3H]D-aspartate from synaptosomes exposed to metabolic inhibitors¹³⁵) but there remains a possible problem with the interpretation of the data obtained *in vivo*.¹³⁴) Specifically, it may not be possible to estimate reliably the inhibitor concentrations directly in the vicinity of the transporters and, as DHK is not absolutely transporter-selective,¹¹⁰) the specific role of GLT in the process may have to be verified using additional, more potent and selective inhibitors, as they become available.

Perhaps, the reversed GluT is the main mechanism of glutamate release in severe ischaemia,¹³¹) but other mechanisms such as the Ca^{2+} -sensitive release from nerve endings or the passage through volume-sensitive channels in swollen cells may also contribute to the release of L-glutamate.^{133,134,136}) One might then speculate whether the latter mechanisms would not be more important—relative to the reversed GluT—in the less severely affected tissue such as that found on, or close to, the periphery of the stroke-affected brain area (penumbra). The penumbra is characterized by delayed neuronal death thus leaving hope to clinicians and medicinal chemists that a therapy would one day be developed that could rescue the afflicted neurons and limit the ischaemic damage. If the reversal of GluT were indeed of secondary importance in the penumbra, trying to use GluT inhibitors to rescue the neurons might not be the right idea. Reduced GluT indeed increases—rather than limits—the damage in

brain tissue exposed to ischemia or experimental injury.^{137,138})

Furthermore, it has not been satisfactorily established that the high extracellular concentrations of L-glutamate, as measured in various experimental models, reflect the actual concentrations of Glu in the vicinity of the neuronal membranes or, indeed, whether the increased concentrations of Glu can be unequivocally causally related to the neuronal loss following the ischaemic injury to the brain tissue.^{136,139,140}) L-Glutamate acts on many receptors (GluR); some of them may mediate neurotoxicity, others could get rapidly desensitised or even have a neuroprotective effect^{141,142}) (for a review see¹⁴³) while the activation of a certain subtype of NMDA receptors (a class of GluR often linked with neurotoxicity of Glu) might trigger off an opening of the blood brain barrier thus allowing the passage of additional potential neurotoxins from the blood stream into the brain interstitial space.¹⁴⁴) The problem of L-glutamate-caused damage in the brain ischaemia is, therefore, quite complex and does not easily lend itself to simple explanations.

Slow Neurodegeneration in ALS—Does Defective GluT Influence Energy Metabolism? Possible involvement of Glu neurotoxicity in the slow neurodegenerative disease is not simple, either. As mentioned earlier, GluT has been considered in the mechanism of ALS and, indeed, severely decreased levels of GLT in spinal cords of patients dying from ALS have been reported.¹⁴⁵) Neither the GLT knockout mice nor rats after GLT antisense knockout *in vivo* have, however, shown any clear signs of developing ALS^{125,146–149}) possibly because of compensatory changes in other components of glutamatergic synapses (in the knockout mice) or because the antisense knockout (in adult rats) was not complete and/or did not last long-enough to have an effect. More recently, GLT has been reported to be reduced in an experimental model of ALS.¹⁵⁰) The particular animal model was based on an expression of a mutant form of Cu^{2+} - and Zn^{2+} -dependent superoxide dismutase (Cu^{2+} , Zn^{2+} -SOD) that has been associated with FALS (a familial form of ALS in humans). The mutant (Cu^{2+} , Zn^{2+} -SOD) retains its enzyme activity but it can decrease the activity of GluT when co-expressed with GLT in *Xenopus laevis* oocytes.¹⁵¹) It has been suggested, therefore, that the decrease in GLT is not the primary cause of ALS.¹⁵⁰) It might, however, be a “common denominator” of various mechanisms leading to the loss of motor neurons and as such it would still provide a convenient target in the development of neuroprotective therapies, particularly in the ALS. However—could a gradual and, initially, perhaps quite a small decrease in GluT activity indeed raise L-glutamate to neurotoxic levels? Administration of GluT inhibitors *in vivo* does not necessarily result in neurodegeneration^{139,152}); in fact, inhibition of GluT that is neurotoxic under conditions in culture^{155–158}) only seems to have neurotoxic consequences *in vivo* when it is combined with a disruption of energy metabolism,^{159,160}) or, when the GluT inhibition continues for many days, such as when L-*t*PDC is chronically infused into the brain.¹⁶³) Additional mechanisms must, therefore, be considered.

Metabolism of L-glutamate in the CNS forms part of a “glutamate-glutamine” cycle^{163–165}) that is compartmentalised between neurons and glia.^{17,166,167}) GluT, in the form of GLT located in the astrocytes in the vicinity of glutamatergic

gic synapses, would seem to provide a crucial mechanism not only for the removal of L-glutamate from the synaptic cleft but also for the supply of L-glutamate into the glial metabolism. Perturbed metabolism in the glial cells may result, for example, in a decrease in the amount of lactate supplied to neurons where it is normally used as a source of energy.¹²⁵⁾ Changes in the metabolism following an inhibition of GluT in brain tissue *in vitro* have been demonstrated^{106,125)} and the possibility thus remains that a progressive decrease in the activity of GluT during the development of ALS can cause a gradual disruption in the glial and neuronal metabolism that might "exhaust" the neurons and make them more vulnerable to neurotoxic insults. The hypothesis is compatible with the slow neurodegeneration observed in the ALS where the motor neurons could be dying over a period of many days and weeks.

Alzheimer Disease Possible existence of altered or defective GluT in brains of patients with Alzheimer disease (AD) has also been discussed.^{168–171)} Immunocytochemical studies have, however, failed to identify any definite specific changes in the transporter abundance or distribution that would correlate with the presence or severity of AD¹⁷²⁾ (for more detailed discussion and additional references see ref.¹⁷⁾). Based on the current state of knowledge, aetiology of AD appears to be quite a complex process and the possibility that changes in GluT contribute to it cannot be simply ruled out. The specificities of the pathological mechanisms associated with the altered GluT in AD¹⁶⁹⁾, however, remain to be elucidated.

Glutamate Hypothesis of Schizophrenia Sheer numbers of glutamate-releasing nerve terminals coupled with the ubiquity and variety of glutamate receptors and glutamate transporters in the CNS imply an enormous importance of glutamatergic synaptic signalling for the normal function of brain. Evidently, the extent and complexity of the system must also mean that there are great many opportunities for things going wrong. Such multitude of possible breakdowns should have been translated into a multitude of novel ideas explaining mechanisms of mental disorders but, in this respect, the potential of the glutamatergic system in the CNS does not yet seem to have been fully exploited by biological psychiatrists. Nevertheless, a distinct "glutamate hypothesis of schizophrenia" has been put forward.^{173,174)} Whereas an increased excitation by L-glutamate seems to be central to the aetiology of the neurodegenerative disorders, in schizophrenia it is an inadequate function of glutamatergic systems that has been proposed as being at fault; see ref.^{173,174)} for reviews. For example, glutamatergic neurotransmission could be weakened by an overactive GluT and, in such context, it may not be without interest to note that among the first drugs shown to inhibit GluT was chlorpromazine.¹⁹⁾ An inhibition of NMDA receptors has been reported to cause schizophrenia-like behavioural disturbances and these effects can be reversed by neuroleptics.¹⁷⁵⁾ In the central nervous tissue of patients with schizophrenia the glutamate receptors may not be "inhibited" though; only the glutamatergic system may function in an inadequate manner.¹⁷³⁾ The explanation of the actions of neuroleptics would therefore require an alternative mechanism for the potentiation of the synaptic actions of L-glutamate. Inhibiting or downregulating of GluT would seem to fit the description of such hypothetical mechanism and, in-

deed, the opposite effect produced by neuroleptics, specifically, the downregulation of GluT by haloperidol and clozapine, has now been documented experimentally.^{176,177)} One cannot help wondering whether GluT should not be considered an ideal target for a new generation of more powerful and more specific neuroleptics!

Can Manipulation of GluT Be Beneficial in Diseases of the CNS? In short, much research remains to be done before the conditions, under which non-transportable inhibitors of GluT could be of benefit in brain ischaemia, are defined, that is to say, if such conditions exist at all. In the case of mental disorders such as schizophrenia, there are indications in the literature that downregulation or inhibition of GluT might be of some therapeutic use in future, while the problem in chronic neurodegenerative diseases (ALS) would seem to be quite different—can the restoration of the normal function (upregulation) of GluT help to reduce the extracellular levels of L-glutamate and/or stabilize the metabolism thus rescuing the neurons from slowly dying away?

The regulation of GluT expression in brain cells is one promising direction of research that is not being addressed in this review (see ref.¹⁷⁸⁾ for a recent summary), instead, the next section deals with issues that might be of more direct importance to pharmacologists and medicinal chemists in the coming years.

UNRESOLVED PROBLEMS AND FUTURE DIRECTIONS

Protein Chemistry Even after nearly 10 years of working with cloned glutamate transporters, the exact nature and positioning of the substrate-binding sites on the transporter molecules is only poorly understood. As pointed out earlier, there has been a wealth of information on the substrate specificity and structural requirements of GluT^{19,26,34,92–101,103,105,107–109)} even in relation to individual EAAT's^{110,112,114,179–183)} yet this structure-activity data has not so far been satisfactorily matched with the protein architecture of the putative substrate-binding regions. Moreover, some of the EAAT's may form multimeric complexes¹⁸⁴⁾ and most of them also act as chloride channels¹⁸⁵⁾; for a review see ref.¹⁶⁾ While Na⁺ is stoichiometrically co-transported down the concentration gradient thus yielding free energy that can be used in the translocation of the substrate (L-glutamate) against a concentration gradient (for further details see e.g. ref.¹⁸⁶⁾ there is no direct thermodynamical link with the movement of Cl[−] (chloride ions follow the concentration gradient downwards *i.e.* they are not actively transported).

The protein structure of the glutamate transporters¹⁸⁷⁾ has been extensively discussed in recent publications^{17,188–192)} and only selected aspects of it will be briefly mentioned here, solely to illustrate the nature of the problem. Glutamate transporters are cytoplasmic membrane-associated proteins displaying a number of putative trans-membrane domains (TMD's) and one or two putative re-entrant loops.^{190,192)} Both N-terminal and C-terminal appear to be within the cytoplasm. There are six putative TMD's with probable α -helical arrangements that are located in the part of the molecule closer to the N-terminal but the folding of the remaining part of the amino acid chain has not been described by a universally accepted model. It is agreed that there is a long intracel-

lular chain at the C-terminal but the structure of the putative TMD8 poses problems since the composition of the segment in terms of amino acid residues is not thought to be easily conducive to the formation of a typical hydrophobic membrane-spanning α -helix. Yet various studies using single-site mutagenesis and related techniques strongly implicate this part of the molecule in the mechanisms of binding and translocation of the substrate. Arrangement of the amino acid sequence into an α -helix with one hydrophilic and one hydrophobic side would seem to be an elegant way around the problem, provided that the hydrophilic part could face (an)other such structure(s) and result in the formation of an aqueous pore, that is perhaps the most characteristic feature of the EAAT family of transporters. The putative TMD7 of the GLT model would seem to be an obvious candidate for such a TMD8-complementary structure. Indeed, TMD7 is overlapped by a sequence that may be responsible for the affinity of GLT for dihydrokainate¹⁹³) and has to be, therefore, considered as being more or less directly involved in the recognition of substrate, providing at least a circumstantial argument in favour of TMD7/TMD8 interaction. How would, however, the pore be formed in GLAST, when the current model of GLAST folding does not allow for the formation of TMD7¹⁹²)? There, must, therefore, be other parts of the GLAST molecule that would participate in the transport process *e.g.* by influencing the affinity for particular substrates (R122 and R280 near TMD3 and TMD5, respectively, for a review see ref.¹⁷) but any involvement of such structures in the formation of the hypothetical aqueous pores must be considered entirely speculative at this stage. Perhaps, a functional model involving multimeric complexes of transporters¹⁸⁴) could bring a solution.

The binding of Na^+ requires the GLT residues 396 to 400 while the residues 403 and 404 seem to be involved in the interaction with K^+ ^{194–196}) but what is the exact position of the binding sites for L-glutamate? Does the substrate bind only to one site or does it bind sequentially to several sites, perhaps as the protein undergoes conformational changes in response to the binding of Na^+ ? Since the passage of Cl^- through the transporters can also have physiological significance^{197–199}) can (a) ligand(s) be found that would inhibit L-glutamate transport but not the chloride channel properties of the transporters? Medicinal chemists wishing to design pharmaceuticals that would act by specifically influencing L-glutamate transport will certainly benefit when answers to such questions have been found.

Another interesting problem is the presence of “scaffolding” proteins that have been shown to influence the function of the transporters in the salamander retina.²⁰⁰) Transporter-associated proteins (TAP’s) have been recently identified in the mammalian brain too,^{201,202}) and, even though their function has not yet been elucidated, in view of possible allosteric effects on the substrate- or Na^+ -binding sites, both their structures and the nature of their interaction with the transporters could be just as important for the physiology and pharmacology of GluT as the characteristics of the transporters themselves.

GluT and Quantitative Autoradiography of [^3H]L- and [^3H]D-Aspartate Binding Recently summarised data on [^3H]L-aspartate binding studied by autoradiographical techniques have also raised some interesting questions. As men-

tioned earlier, Na^+ -dependent binding of [^3H]D-aspartate to the sections of frozen rat brain was introduced many years ago as a means to study and quantify the regional distribution of GluT.^{57,58}) The sections are labelled by a suitable radioligand *in vitro* and subsequently exposed against ^3H -sensitive film. The resulting image can be densitometrically analysed and the binding expressed as an amount of ^3H bound per unit of brain tissue.^{63,67}) [^3H]D-Aspartate binding studied in such fashion was reported not to decrease in parts of hippocampus following lesions to glutamatergic pathways terminating in the area⁶⁰) and this finding has undoubtedly contributed to arguments against the Na^+ -dependent binding of [^3H]D-aspartate being a marker for GluT. It is now known, however, that some of the transporters, particularly GLT and GLAST are located in astrocytes and, even if some reduction in the glial expression of GLT may have taken place in the area following a decrease in glutamatergic input,^{203,204}) it may not have caused great-enough changes in the Na^+ -dependent binding of [^3H]D-aspartate (that could represent binding to several transporters, not just GLT) to be detected by the techniques used at the time.

More specific line of criticism of radioligand binding studies on GluT has focussed on possible sequestration of the radioligand by “resealed plasma membrane vesicles”. This would really be equivalent to “uptake” as it has been observed in synaptosomes and brain slices.^{18,19}) The uptake was, however, found to be virtually abolished in the presence of the inhibitor of Na^+ , K^+ -dependent ATPase ouabain.¹⁸) GluT is, of course, driven by Na^+ and K^+ gradients that have to be maintained by ATP-derived energy whereas the labelling of frozen sections takes place at 2 to 4 °C³⁵) and under those conditions no such gradients could form or be maintained for very long periods of time. It has been, furthermore, suggested¹⁷) that the labelling of the sections could take place *via* an “exchange” mechanism¹⁷) thus presumably implying that the tissue that had been frozen, thawed and pre-incubated, with several changes of media,⁶⁴) would still contain significant pools of endogenous L-glutamate and L-aspartate that could not cross the membranes of the hypothetical “resealed plasma membrane vesicles”, except by using GluT in a one-for-one exchange with GluT substrates outside; *cf.* Fig. 5D in ref.¹⁷) Binding of [^3H]D-aspartate to sections of frozen brain tissue actually survives treatment with organic solvents, such as xylene, that should dissolve membrane lipids thus disrupting any osmotically distinct entities that might have existed and/or might have formed in the frozen and thawed tissue⁵⁹) and would have contained any residual “exchangeable” L-glutamate and L-aspartate.

It seems then, that the Na^+ -dependent binding of radioligands to GluT reflects the distribution of binding sites in the fresh-frozen (*i.e.* non-fixed) tissue that behave, in terms of substrate selectivity and ionic dependence, as the substrate-binding sites on GluT and does so in a quantitatively reproducible manner.^{63–67}) It is tempting, therefore, to suggest that the autoradiographical analysis of [^3H]L- or [^3H]D-aspartate binding is in some aspects “better” than the immunocytochemistry which uses fixed tissue, antibodies directed against small parts of selected molecules and cannot easily yield information on biochemistry, pharmacology or ionic dependence of GluT. In contrast, the autoradiography of radioligand binding should produce more faithful (quantitative) pic-

ture of GluT distribution in the tissue and it should be able to describe its characteristics *in situ* at the same time. But is it really so?

The binding of [^3H]L-aspartate rapidly equilibrates⁶⁴⁾ and even though the apparent K_D and B_{max} would be difficult to determine directly because of high concentrations of ^3H -label required to reach saturation, it has been possible to estimate the relative potencies of GluT inhibitors (expressed as IC_{50}) with very high precision, using radioligand displacement studies.²⁰⁵⁾ Such constants are, of course, determined at apparent equilibrium and must be considered distinct from the similar constants obtained when the kinetics of the radioligand uptake is studied. The rate constants of association and dissociation, which determine the binding affinity of GluT substrates or GluT inhibitors are, nevertheless, important in both types of experiments (the binding of the substrate to the GluT is, in fact, very important for the kinetics of the GluT in the synaptic environment^{206–208)}). One would, therefore, expect a degree of correspondence between the values of IC_{50} (or K_i) as determined in kinetic experiments with [^3H]L-glutamate uptake in brain slices^{19,26,99)} and the values of IC_{50} (or K_i) obtained in binding studies (Na^+ -dependent [^3H]L-aspartate binding in sections of frozen brain). In reality, this is almost precisely what has been observed.¹³⁾ Specifically, in both experimental models, L- and D-aspartate,^{13,19)} L- and D- tBOHA ^{13,26)} and L-cysteinesulphonate¹⁹⁾ (Takamoto, Balcar and Yoneda, unpublished autoradiographical studies) are strong inhibitors, kainate, DHK,^{13,99)} DL-2-aminoadipate, DL-3-aminoadipate and D-glutamate^{13,19)} are weak or inactive and L-serine-*O*-sulphate is of about intermediate potency as an inhibitor of either the uptake,¹⁹⁾ or binding.¹³⁾ There is one clear exception though; it is L-glutamate. While in the experiments in brain slices, L-glutamate is approximately equipotent with L- and D-aspartate,¹⁹⁾ in the binding studies it displays about 50 times lower affinity for the binding sites than either D- or L-aspartate.¹³⁾ The other interesting observation is that, in the case of [^3H]L-aspartate binding, there is a significant difference between the forebrain and the cerebellar cortex in the affinities of at least three ligands: D-aspartate, L- tPDC and L- tBOHA .¹³⁾

Some of the findings could, perhaps, be explained by unusual folding of the transporter molecules in membranes that had been subjected to freezing and thawing and were used in binding experiments at 2–4 °C.^{64–67)} Alternatively, the “unusual” folding may simply reflect the normal conformation of the transporter protein in the plasma membrane of brain cells as opposed to the plasma membranes of *Xenopus laevis* oocytes where many of the structure–activity studies on individual transporters have been done (*cf.* ref.¹¹⁰⁾ for a review).

In fact, it is not only the folding, presence/absence of TAP's/scaffolding proteins and formation of multimeric complexes that have to be taken into account when considering the differences between EAAT's expressed in artificial systems and those present in brain. The central nervous tissue has a particularly strong propensity for alternative splicing²⁰⁹⁾ and the occurrence of unusual splice variants may explain the characteristics of the [^3H]L-aspartate and [^3H]D-aspartate binding in brain sections.^{13,64–67)} The regional distribution of [^3H]L-aspartate and [^3H]D-aspartate binding (cerebellar cortex forebrain) is similar to that of GLAST (EAAT1) and a GLAST variant that prefers L- over D-aspartate—in analogy

to the [^3H]L-aspartate and [^3H]D-aspartate binding site in the rat cerebellar cortex^{13,63)}—has recently been cloned from an insect nervous system.²¹⁰⁾ Furthermore, a Na^+ -dependent uptake system that strongly prefers D-aspartate to L-glutamate has been reported in cultured glioma cells.⁹⁴⁾ Thus [^3H]L-aspartate and [^3H]D-aspartate may selectively bind to particular GLAST variants and may not at all universally label all GluT sites in the tissue. The visualization of the distribution of GluT sites by autoradiography of [^3H]L-aspartate and [^3H]D-aspartate binding is, therefore, no more “faithful” than the images obtained by immunocytochemistry. Evidently, radioligand binding labels a specific subset of GluT sites, depending on what radiolabelled substrate or inhibitor is used.

GluT in Nerve Endings—the Last Frontier? Perhaps the most puzzling unsolved problem of the molecular biology and neurochemistry of GluT is the presence of GluT in synaptic nerve endings. When the uptake of [^3H]L-glutamate and [^3H]L-aspartate was first studied in brain homogenates more than 30 years ago it seems to have been virtually assumed that it would be located primarily in synaptosomes or “pinched-off nerve endings (see¹³⁾ for a historical perspective). More recent evidence, however, has been controversial. As mentioned earlier, GLT mRNA has been identified in neurons, but immunocytochemistry has failed to detect GLT—or any other known EAAT—in nerve endings of the normal adult brain. Yet the nerve endings in the CNS do accumulate D-aspartate by a Na^+ -dependent process^{211,212)} and synaptosomal fractions prepared from brains of GLT-knock-out mice do display decreased uptake of [^3H]L-glutamate¹⁴⁷⁾ (there is a strong possibility of contamination of synaptosomal fractions by glial material,^{13,75,213–215)} though), so there could be an as yet unidentified EAAT, perhaps a GLT splice variant, located presynaptically.^{16,17)} With the advent of substrates selective for individual EAAT's^{111–114)} (*cf.* also Fig. 2) it might yet be possible to produce a radioligand with high affinity for the neuronal GLT and study it by quantitative autoradiography!

CONCLUSION

The sodium dependent transport of L-glutamate in brain tissue has been studied for more than thirty years¹³⁾ and there are now many independent lines of evidence strongly implicating GluT as the main mechanism regulating the actions of L-glutamate at a great majority of the excitatory synapses in the central nervous system.^{17,216,217)} The transport is mediated by a family of protein molecules displaying extraordinary degree of complexity in both structure and function thus presenting the pharmacologists and medicinal chemists with unusually challenging tasks. The rewards for the solutions, however, are likely to be exceptionally great, too. On available evidence, GluT transport plays fundamental roles in the mechanisms of the most serious and most frequent problems faced by neurologists and psychiatrists: the damage caused to the central nervous system by ischaemia (stroke, cerebral and spinal trauma) and the dying of brain cells in the neurodegenerative diseases. In addition, a possibility seems to be emerging that the pharmacological manipulation of GluT could be helpful in the therapy of schizophrenia. Advances on any of these fronts are likely to be of significant benefit to many patients.

Acknowledgements The author wishes to thank to the Japanese Ministry for Education, Culture, Sports, Science and Technology for funding his position at Kanazawa University and to Professor Yukio Yoneda for his continuing encouragement and valuable comments.

REFERENCES

- 1) Present address: *Department of Anatomy and Histology, Faculty of Medicine, Anderson Stuart Building F13, The University of Sydney NSW 2006.*
- 2) Hayashi T., *Jpn. J. Physiol.*, **3**, 46—64 (1952).
- 3) Hayashi T., *Keio Med. J.*, **3**, 183—192 (1954).
- 4) Curtis D. R., Watkins J. C., *Nature* (London), **191**, 1010—1011 (1961).
- 5) Curtis D. R., Watkins J. C., *J. Neurochem.*, **6**, 102—141 (1960).
- 6) Curtis D. R., Johnston G. A. R., *Rev. Physiol. Biochem. Exp. Pharm.*, **69**, 97—188 (1974).
- 7) Krnjevic K., *Physiol. Rev.*, **54**, 418—540 (1974).
- 8) Roberts P. J., Storm-Mathisen J., Johnston G. A. R., "Glutamate: Transmitter in the Central Nervous System," John Wiley & Sons, Chichester, U.K., 1981.
- 9) Fonnum F., *J. Neurochem.*, **42**, 1—11 (1984).
- 10) Takagaki G., *Neurochem. Internat.*, **29**, 225—229 (1996).
- 11) Lodge D., "The Ionotropic Glutamate Receptors," ed. by Monaghan D. T., Wenthold R. J., Humana Press Inc., Totowa, NJ, U.S.A., 1997, pp. 1—38.
- 12) Bennett M. R., Balcar V. J., *Neurochem. Internat.*, **35**, 269—280 (1999).
- 13) Balcar V. J., Takamoto A., Yoneda Y., *Collect. Czech. Chem. Commun.*, **66**, 1315—1340 (2001).
- 14) Logan W. J., Snyder S. H., *Nature* (London), **234**, 297—299 (1971).
- 15) Wofsey A. R., Kuhar M. J., Snyder S. H., *Proc. Natl. Acad. Sci. U.S.A.*, **68**, 1102—1106 (1971).
- 16) Danbolt N. C., "Glutamate," ed. by Ottersen O. P., Storm-Mathisen J., Vol. 18, *Handbook of Chemical Neuroanatomy*, Series ed. by Björklund A., Hökfelt T., Elsevier, Amsterdam, The Netherlands, 2000, pp. 231—254.
- 17) Danbolt N. C., *Prog. Neurobiol.*, **65**, 1—105 (2001).
- 18) Balcar V. J., Johnston G. A. R., *J. Neurobiol.*, **3**, 295—301 (1972).
- 19) Balcar V. J., Johnston G. A. R., *J. Neurochem.*, **19**, 2657—2666 (1972).
- 20) Iversen L. L., Johnston G. A. R., *J. Neurochem.*, **18**, 1939—1950 (1971).
- 21) Balcar V. J., Johnston G. A. R., *J. Neurochem.*, **20**, 529—539 (1973).
- 22) Henn F. A., Goldstein M. N., Hamberger A., *Nature* (London), **249**, 663—664 (1974).
- 23) Balcar V. J., Borg J., Mandel P., *J. Neurochem.*, **28**, 87—93 (1977).
- 24) Schousboe A., Svenneby G., Hertz L., *J. Neurochem.*, **29**, 999—1005 (1977).
- 25) Schousboe A., *Int. Rev. Neurobiol.*, **22**, 1—45 (1981).
- 26) Balcar V. J., Johnston G. A. R., Twitchin B., *J. Neurochem.*, **28**, 1145—1146 (1977).
- 27) Johnston G. A. R., Lodge D., Bornstein J. C., Curtis D. R., *J. Neurochem.*, **34**, 241—243.
- 28) Lucas D. R., Newhouse J. P., *A.M.A. Arch. Ophthalmol.*, **58**, 193—204 (1957).
- 29) Olney J. W., Sharpe L. G., Feigin R. D., *J. Neuropath. Exp. Neurol.*, **31**, 464—488 (1972).
- 30) McBean G. J., Roberts P. J., *J. Neurochem.*, **44**, 247—254 (1985).
- 31) Gordon R. D., Balazs R., *J. Neurochem.*, **40**, 1090—1099 (1983).
- 32) Ferkany J., Coyle J. T., *J. Neurosci. Res.*, **16**, 491—503 (1986).
- 33) Robinson M. B., Hunter-Ensor M., Sinor J., *Brain Res.*, **544**, 196—202 (1991).
- 34) Balcar V. J., Schousboe A., Spoerri P. E., Wolff J. R., *Neurochem. Internat.*, **10**, 213—217 (1987).
- 35) Balcar V. J., Li Y., *Life Sci.*, **51**, 1467—1478 (1992).
- 36) Pines G., Danbolt N. C., Björås M., Zhang Y., Bendahan A., Eide L., Koepsel H., Storm-Mathisen J., Seeberg E., Kanner B. I., *Nature* (London), **360**, 464—467 (1992).
- 37) Kanai Y., Hediger M. A., *Nature* (London), **360**, 467—471 (1992).
- 38) Storck T., Schulte S., Hoffmann K., Stoffwel W., *Proc. Natl. Acad. Sci. U.S.A.*, **89**, 10955—10959 (1992).
- 39) Tanaka K., *Neurosci. Lett.*, **159**, 183—186 (1993).
- 40) Tanaka K., *Neurosci. Res.*, **16**, 149—153 (1993).
- 41) Fairman W., Vandenberg R. J., Arriza J. L., Kavanaugh M. P., Amara S. G., *Nature* (London), **375**, 599—603 (1995).
- 42) Arriza J. L., Eliasof S., Kavanaugh M. P., Amara S. G., *Proc. Natl. Acad. Sci. U.S.A.*, **94**, 4155—4160 (1997).
- 43) Robinson M. B., *Neurochem. Internat.*, **33**, 479—491 (1998).
- 44) Sims K. D., Robinson M. B., *Crit. Rev. Neurobiol.*, **13**, 169—197 (1999).
- 45) Tanaka K., *Neurosci. Res.*, **37**, 15—19 (2000).
- 46) Robinson M. B., Dowd L. A., *Adv. Pharmacol.*, **37**, 69—115 (1997).
- 47) Rothstein J. D., Martin L. J., Levey A. I., Dykes-Hoberg M., Jin L., Wu D., Nash N., Kuncel R. W., *Neuron*, **13**, 713—725 (1994).
- 48) Lehre K. P., Levy L. M., Ottersen O. P., Storm-Mathisen J., Danbolt N. C., *J. Neurosci.*, **15**, 1835—1853 (1995).
- 49) Schmitt A., Asan E., Püschel B., Jöns T., Kugler P., *Neurosci.*, **71**, 989—1004 (1996).
- 50) Schmitt A., Asan E., Püschel B., Kugler P., *J. Neurosci.*, **17**, 1—10 (1997).
- 51) Danbolt N. C., Lehre K. P., Dehnes Y., Chaudry F. A., Levy L. M., *Methods Enzymol.*, **296**, 388—407, (1998).
- 52) Danbolt N. C., Chaudry F. A., Dehnes Y., Lehre K. P., Levy L. M., Ullensvang K., Storm-Mathisen J., *Prog. Brain Res.*, **116**, 23—43 (1998).
- 53) Lehre K. P., Danbolt N. C., *J. Neurosci.*, **18**, 8751—8757 (1998).
- 54) Kugler P., Schmitt A., *Glia*, **27**, 129—142 (1999).
- 55) Young W. S. 3rd, Kuhar M. J., *Brain Res.*, **28**, 255—270 (1979).
- 56) Greenamyre J. T., Young A. B., Penney J. B., Jr., *J. Neurosci.*, **4**, 2133—2144 (1984).
- 57) Parsons B., Rainbow T. C., *Neurosci. Lett.*, **36**, 9—12 (1983).
- 58) Anderson K. J., Monaghan D. T., Bridges R. J., Tavoularis A. L., Cotman C. W., *Neuroscience*, **38**, 311—322 (1990).
- 59) Anderson K. J., Vickroy T. W., *Eur. J. Pharmacol.*, **185**, 119—121 (1990).
- 60) Greenamyre J. T., Higgins D. S., Young A. B., *Brain Res.*, **511**, 310—318 (1990).
- 61) Anderson K. J., Bridges R. J., Cotman C. W., *Brain Res.*, **562**, 285—290 (1991).
- 62) Anderson K. J., Sandler D. L., *Life Sci.*, **52**, 863—868 (1993).
- 63) Takamoto A., Quiggin L. B., Lieb I., Shave E., Balcar V. J., Yoneda Y., *Life Sci.*, **70**, 991—1001 (2002).
- 64) Li Y., Balcar V. J., *Exp. Brain Res.*, **97**, 415—422 (1994).
- 65) Balcar V. J., Li Y., Killinger S., *Neurochem. Internat.*, **26**, 155—164 (1995).
- 66) Killinger S., Blume G. L., Bohart L., Bested A., Dias L. S., Cooper B., Allan R. D., Balcar V. J., *Neurosci. Lett.*, **216**, 101—104 (1996).
- 67) Lieb I., Chebib M., Cooper B., Dias L. S., Balcar V. J., *Neurochem. Internat.*, **36**, 319—327 (2000).
- 68) Thorp R., Danbolt N. C., Babaie E., Björås M., Seeberg E., Storm-Mathisen J., Ottersen O. P., *Eur. J. Neurosci.*, **6**, 936—942 (1994).
- 69) Thorp R., Hoover F., Danbolt N. C., Storm-Mathisen J., Ottersen O. P., *Anat. Embryol.*, **195**, 317—326 (1997).
- 70) Berger U. V., Hediger M. A., *Anat. Embryol.*, **198**, 13—30 (1998).
- 71) Haugeto Ø., Ullensvang K., Levy L. M., Chaudry F. A., Honoré T., Nielsen M., Lehre K. P., Danbolt N. C., *J. Biol. Chem.*, **271**, 27715—27722 (1996).
- 72) Perego C., Vanoni C., Bossi M., Massari S., Basudev H., Longhi R., Pietrini G., *J. Neurochem.*, **75**, 1076—1084 (2000).
- 73) Zhou M., Kimelberg H., *J. Neurosci.*, **21**, 7901—7908 (2001).
- 74) Kanai Y., Bhide P. G., DiFiglia M., Hediger M. A., *Neuroreport*, **6**, 2357—2362 (1995).
- 75) Dowd L. A., Coyle A. J., Rothstein J. D., Pritchett D. B., Robinson M. B., *Mol. Pharmacol.*, **49**, 465—473 (1996).
- 76) Dunlop J., Lou Z., McIlvain H. B., *Brain Res.*, **839**, 235—242 (1999).
- 77) Palos T. P., Ramachandran B., Boado R., Howard B. D., *Brain Res., Mol. Brain Res.*, **37**, 297—303 (1996).
- 78) Furuta A., Martin L. J., Lin C. L. G., Dykes-Hoberg M., Rothstein J. D., *Neuroscience*, **81**, 1031—1042 (1997).
- 79) Furuta A., Rothstein J. D., Martin L. J., *J. Neurosci.*, **17**, 8363—8375 (1997).
- 80) Brooks-Kayal A. R., Munir M., Jin H., Robinson M. B., *Neurochem. Int.*, **22**, 95—100 (1998).
- 81) Mennerick S., Dhond R. P., Bencz A., Xu W. Y., Rothstein J. D., Dan-

- bolt N. C., Isenberg K. E., Zorumski C. F., *J. Neurosci.*, **18**, 4490—4499 (1998).
- 82) Stanimirovic D. B., Ball R., Small D. L., Muruganadam A., *J. Dev. Neurosci.*, **17**, 173—184 (1999).
 - 83) Plachez C., Danbolt N. C., Recasens M., *J. Neurosci. Res.*, **59**, 587—593 (2000).
 - 84) Meaney J. A., Balcar V. J., Rothstein J. D., Jeffrey P. L., *J. Neurosci. Res.*, **54**, 595—603 (1998).
 - 85) Gaillat S., Plachez C., Malaval F., Bezine M. F., Recasens M., *Neurochem. Internat.*, **38**, 293—301 (2001).
 - 86) Northington F. J., Traystman R. J., Koehler R. C., Martin L. J., *J. Neurobiol.*, **39**, 515—526 (1999).
 - 87) Sutherland M. L., Delaney T. L., Noebels J. L., *J. Neurosci.*, **16**, 2191—2207 (1996).
 - 88) Rauen T., *Amino Acids* **19**, 53—62 (2000).
 - 89) Pow D. V., *Neurochem. Internat.*, **38**, 463—484 (2001).
 - 90) Eliasof S., Arriza J. L., Leighton B. H., Kavanaugh M. P., Amara S. G., *J. Neurosci.*, **18**, 698—712 (1998).
 - 91) Christensen H. N., Greene A. A., Kakuda D. K., MacLeod C. L., *J. Exp. Biol.*, **196**, 297—305 (1994).
 - 92) Cooper B., Chebib M., Shen J., King N. J. C., Darvey I. G., Kuchel P. W., Rothstein J. D., Balcar V. J., *Arch. Biochem. Biophys.*, **353**, 356—364 (1998).
 - 93) Balcar V. J., Borg J., Robert J., Mandel P., *J. Neurochem.*, **34**, 1678—1681 (1980).
 - 94) Erecinska M., Troeger M. B., Wilson D. F., Silver I. A., *Brain Res.*, **369**, 203—214 (1986).
 - 95) Roberts P. J., Watkins J. C., *Brain Res.*, **85**, 120—125 (1975).
 - 96) Drejer J., Larson O. L., Schousboe A., *Exp. Brain Res.*, **47**, 259—269 (1982).
 - 97) Robinson M. B., Sinor J. D., Dowd L. A., Kerwin J. F., Jr., *J. Neurochem.*, **60**, 167—179 (1993).
 - 98) Garlin A. B., Sinor A. D., Sinor J. D., Jee S. H., Grinspan J. B., Robinson M. B., *J. Neurochem.*, **64**, 2572—2580 (1993).
 - 99) Johnston G. A. R., Kennedy S. M. F., Twitchin B., *J. Neurochem.*, **32**, 121—127 (1979).
 - 100) Bridges R. J., Stanley M. S., Anderson M. W., Cotman C. W., Chamberlin A. R., *J. Med. Chem.*, **34**, 717—725 (1991).
 - 101) Balcar V. J., *FEBS Lett.*, **300**, 203—207 (1992).
 - 102) Pellicciari R., Natalini B., Marinozzi M., Selvi L., Chiorri C., Monahan J. B., Lanthorn T. H., Snyder J. P., "Frontiers in Excitatory Amino Acid Research," ed. by Cavalheiro E. A., Lehman L., Turski L., Alan Liss Inc., New York, NY, U.S.A., 1988, pp. 67—70.
 - 103) Shimamoto K., Ishida M., Shinozaki H., Ohfune Y., *J. Org. Chem.*, **56**, 4167—4176 (1991).
 - 104) Fowden L., Smith A., Millington D. S., Sheppard R. C., *Phytochem.*, **6**, 437—443 (1969).
 - 105) Nakamura Y., Kataoka Y., Ishida M., Shinozaki H., *Neuropharmacol.*, **32**, 833—837 (1993).
 - 106) Moussa C. E.-H., Mitrovic A. D., Vandeberg R. J., Provis T., Rae C., Bubbs W. A., Balcar V. J., *Neurochem. Res.*, **27**, 27—35 (2002).
 - 107) Allan R. D., Hanrahan J. R., Hambley T. W., Johnston G. A. R., Mewett K. N., Mitrovic A. D., *J. Med. Chem.*, **33**, 2905—2910 (1990).
 - 108) Fletcher E. J., Mewett K. N., Drew C. A., Allan R. D., Johnston G. A. R., *Neurosci. Lett.*, **121**, 133—135 (1991).
 - 109) Bridges R. J., Lovering F. E., Koch H., Cotman C. W., Chamberlin A. R., *Neurosci. Lett.*, **174**, 191—197 (1994).
 - 110) Bridges R. J., Kavanaugh M. P., Chamberlin A. R., *Curr. Pharm. Des.*, **5**, 363—379 (1999).
 - 111) Bräuner-Osborne H., Hermit M. B., Nielsen B., Krogsgaard-Larsen P., Johansen T. N., *Eur. J. Pharmacol.*, **406**, 41—44 (2000).
 - 112) Vandenberg R. J., Mitrovic A. D., Chebib M., Balcar V. J., Johnston G. A. R., *Mol. Pharmacol.*, **51**, 809—815 (1997).
 - 113) Apricó K., Beart P. M., Lawrance A. J., Crawford D., O'Shea R. D., *J. Neurochem.*, **77**, 1218—1225 (2001).
 - 114) Eliasof S., McIlvain H. B., Petroski R. E., Foster A. C., Dunlop J., *J. Neurochem.*, **77**, 550—557 (2001).
 - 115) Shimamoto K., Lebrun B., Yasuda-Kamatani Y., Sakaitani M., Shigeri Y., Yumoto N., Nakajima T., *Mol. Pharmacol.*, **53**, 195—201 (1998).
 - 116) Shimamoto K., Shigeri Y., Yasuda-Kamatani Y., Sakaitani M., Lebrun B., Yumoto N., Nakajima T., *Bioorg. Med. Chem. Lett.*, **10**, 2407—2410 (2000).
 - 117) Anderson C. M., Bridges R. J., Chamberlin A. R., Shimamoto K., Yasuda-Kamatani Y., Swanson R. A., *J. Neurochem.*, **79**, 1207—1216 (2001).
 - 118) Jabaudon D., Scanziani M., Gähwiler B. H., Gerber U., *Proc. Natl. Acad. Sci. U.S.A.*, **97**, 5610—5615 (2000).
 - 119) Phillis J. W., Ren J., O'Regan M. H., *Brain Res.*, **868**, 105—112 (2000).
 - 120) Waagepetersen H. S., Shimamoto K., Schousboe A., *Neurochem. Res.*, **26**, 661—666 (2001).
 - 121) Shigeri Y., Shimamoto K., Yasuda-Kamatani Y., Seal R. P., Yumoto N., Nakajima T., Amara S. G., *J. Neurochem.*, **79**, 297—302 (2001).
 - 122) Bennett J. P., Jr., Logan W. J., Snyder S. H., *J. Neurochem.*, **21**, 1533—1550 (1973).
 - 123) Johnston G. A. R., "Glutamate: Transmitter in the Central Nervous System," ed. by Roberts P. J., Storm-Mathisen J., Johnston G. A. R., John Wiley & Sons, Chichester, U.K., 1981, pp. 77—87.
 - 124) Rothstein J. D., Martin L. J., Kuncel R. W., *N. Eng. J. Med.*, **326**, 1464—1468 (1992).
 - 125) Rae C., Lawrance M. L., Dias L. S., Provis T., Bubbs W. A., Balcar V. J., *Brain Res. Bull.*, **53**, 373—381 (2000).
 - 126) Sánchez-Prieto J., González P., *J. Neurochem.*, **50**, 1322—1324 (1988).
 - 127) Swanson R. A., *Neurosci. Lett.*, **147**, 143—146 (1992).
 - 128) O'Neill C. M., Ball S. G., Vaughan P. F., *J. Neurochem.*, **63**, 603—611 (1994).
 - 129) Szatkowski M., Barbour B., Attwell D., *Nature (London)*, **348**, 443—446 (1990).
 - 130) Szatkowski M., Attwell D., *Trends in Neurosci.*, **17**, 359—365 (1994).
 - 131) Rossi D. J., Oshima T., Attwell D., *Nature (London)*, **403**, 316—321 (2000).
 - 132) Longuemare M. C., Rose C. R., Farrell K., Ransom B. R., Waxman S. G., Swanson R. A., *Neuroscience*, **93**, 285—292 (1999).
 - 133) Anderson C. M., Swanson R. A., *Glia*, **32**, 1—14 (2000).
 - 134) Seki Y., Feustel P. J., Keller R. W., Tranmer B. I., Kimelberg H. K., *Stroke*, **30**, 433—440 (1999).
 - 135) Koch H. P., Chamberlin A. R., Bridges R. J., *Mol. Pharmacol.*, **55**, 1044—1048 (1999).
 - 136) Nishizawa Y., *Life Sci.*, **15**, 369—381 (2001).
 - 137) Rao V. L., Dogan A., Todd K. G., Bowen K. K., Kim B. T., Rothstein J. D., Dempsey R. J., *J. Neurosci.*, **21**, 1876—1883 (2001).
 - 138) Rao V. L., Dogan A., Bowen K. K., Todd K. G., Dempsey R. J., *Eur. J. Neurosci.*, **13**, 119—128 (2001).
 - 139) Obrenovitch T. P., Urenjak J., *Prog. Neurobiol.*, **51**, 39—87 (1997).
 - 140) Obrenovitch T. P., Urenjak J., Zilkha E., Jay T. M., *Int. J. Neurosci.*, **18**, 281—287 (2000).
 - 141) Bruno V., Copani A., Gataglia G., Raffaele R., Shinozaki H., Nicoletti F., *Eur. J. Pharmacol.*, **256**, 109—112 (1994).
 - 142) Lin S.-H., Maiese K., *J. Cerebr. Blood Flow Met.*, **21**, 262—275 (2001).
 - 143) Ozawa S., Kamyia H., Tsuzuki K., *Prog. Neurobiol.*, **54**, 581—618 (1997).
 - 144) Pliss L., Ježová D., Mareš V., Balcar V. J., Štátný F., *Neurosci. Lett.*, **317**, 85—88 (2001).
 - 145) Rothstein J. D., Van Kamen M., Levey A. I., Martin L. J., Kuncel R. W., *Ann. Neurol.*, **38**, 73—84 (1995).
 - 146) Peghini P., Janzen J., Stoffel W., *EMBO J.*, **16**, 3822—3832 (1997).
 - 147) Tanaka K., Watase K., Manabe T., Yamada K., Watanabe M., Takahashi K., Iwama H., Nishikawa T., Ichihara N., Kikuchi T., Okuyama S., Kawashima N., Hori S., Takimoto M., Wada K., *Science*, **276**, 1699—1702 (1997).
 - 148) Watase K., Hashimoto K., Kano M., Yamada K., Watanabe M., Inoue Y., Okuyama S., Sakagawa T., Ogawa S., Kawashima N., Hori S., Takimoto M., Wada K., Tanaka K., *Eur. J. Neurosci.*, **10**, 976—988 (1998).
 - 149) Rothstein J. D., Dykes-Hoberg M., Pardo C. A., Bristol L. A., Jin L., Kuncel R. W., Kanai Y., Hediger M. A., Wang Y., Schielke J. P., Welty D. F., *Neuron*, **16**, 675—686 (1996).
 - 150) Bendotti C., Tortarolo M., Suchak S. K., Calvaresi N., Carvelli N., Bastone A., Rizzi M., Rattray M., Mennini T., *J. Neurochem.*, **79**, 737—746 (2001).
 - 151) Trotti D., Danbolt N. C., Brown R. H., Jr., Hediger M. A., *Nature Neurosci.*, **2**, 427—433 (1999).
 - 152) Ong W. Y., Balcar V. J., *J. Brain Res.*, **38**, 317—322 (1997).
 - 153) Massieu L., Morales-Villagrán A., Tapia R., *J. Neurochem.*, **64**,

- 2262—2272 (1995).
- 154) Tapia R., Medina-Ceja L., Pena F., *Neurochem. Int.*, **34**, 23—31 (1999).
 - 155) Blitzblau R., Gupta S., Djali S., Robinson M. B., Rosenberg P. A., *J. Neurosci.*, **8**, 1840—1852 (1996).
 - 156) Okazaki S., Nishida Y., Kawai H., Saito S., *Neurochem. Res.*, **21**, 1201—1207 (1996).
 - 157) Velasco I., Tapia R., Massieu L., *J. Neurosci. Res.*, **44**, 551—561 (1996).
 - 158) Amin N., Pearce B., *Neurochem. Internat.*, **30**, 217—276 (1997).
 - 159) Sanchez-Carbente, M. R., Massieu L., *J. Neurochem.*, **72**, 129—138 (1999).
 - 160) Garcia O., Massieu L., *J. Neurosci. Res.*, **64**, 418—428 (2001).
 - 161) Massieu L., Del Rio P., Montiel T., *Neuroscience*, **106**, 669—677 (2001).
 - 162) Lievens J. C., Dutertre M., Forni C., Salin P., Kerkerian-Le Goff L., *Brain Res.*, **50**, 181—189 (1997).
 - 163) Van den Berg C. J., Garfinkel D., *Biochem. J.*, **123**, 211—218 (1971).
 - 164) Hamberger A. C., Chiang C. H., Nysten E. S., Scheff S. W., Cotman C. W., *Brain Res.*, **168**, 513—530 (1979).
 - 165) McIlwain H., Bachelard H. S., *Biochemistry of the Central Nervous System*, 5th Edition, Churchill-Livingstone, Edinburgh, U.K., 1985.
 - 166) Hertz L., Dringen R., Schousboe A., Robinson S. R., *J. Neurosci Res.*, **57**, 417—428 (1999).
 - 167) Daikhin Y., Yudkoff M., *J. Nutr.*, **130** (4S Suppl), 1026S—1031S (2000).
 - 168) Cross A. J., Slater P., Simpson M., Royston C., Deakin J. F. W., Perry R. H., Perry E. K., *Neurosci. Lett.*, **79**, 213—217 (1987).
 - 169) Scott H. L., Tannenberg A. E. G., Dodd P. R., *J. Neurochem.*, **64**, 2193—2202 (1995).
 - 170) Harris M. E., Wang Y., Pedigo N. W., Jr., Hensley K., Butterfield D. A., Carney J. M., *J. Neurochem.*, **67**, 277—286 (1996).
 - 171) Masliah E., Alford M., DeTeresa R., Mallory M., Hansen L., *Ann. Neurol.*, **40**, 759—766 (1996).
 - 172) Beckström H., Julsrud L., Haugeto Ø., Dewar D., Graham D. I., Lehre K. P., Storm-Mathisen J., Danbolt N. C., *J. Neurosci. Res.*, **55**, 218—229 (1999).
 - 173) Carlsson A., Hansson L. O., Waters N., Carlsson M. L., *Brit. J. Psychiatry*, **174**, 2—6 (1999).
 - 174) Farber N. B., Newcomer J. W., Olney J. W., *Prog. Brain Res.*, **116**, 421—437 (1998).
 - 175) Banerjee S. P., Zuck L. G., Yablonsky-Alter E., Lidsky T. I., *Neuroreport*, **6**, 2500—2504 (1995).
 - 176) Schneider J. S., Wade T., Lidsky T. I., *Neuroreport*, **9**, 133—136 (1998).
 - 177) Melone M., Vitellaro-Zuccarello L., Valejo-Illarramendi A., Perez-Samartin A., Matute C., Cozzi A., Pellegrini-Gianpietro D. E., Rothstein J. D., Conti F., *Mol. Psychiatry*, **6**, 380—386 (2001).
 - 178) Gegelashvili G., Robinson M. B., Trotti D., Rauen T., *Prog. Brain Res.*, **132**, 267—286 (2001).
 - 179) Arriza J. L., Fairman W. A., Wadiche J. L., Murdoch G. H., Kavanaugh M. P., Amara S. G., *J. Neurosci.*, **14**, 5559—5569 (1994).
 - 180) Vandenberg R. J., Mitrovic A. D., Johnston G. A. R., *Brit. J. Pharmacol.*, **123**, 1593—1600, 1998.
 - 181) Ruiz M., Ortega A., *Neuroreport*, **6**, 2041—2044 (1995).
 - 182) Mitrovic A. D., Amara S. G., Johnston G. A. R., Vandenberg R. J., *J. Biol. Chem.*, **273**, 14698—14706 (1998).
 - 183) Campiani G., De Angelis M., Armaroli S., Fattorusso C., Catalanotti B., Ramunno A., Nacci V., Novellino E., Grewer C., Ionescu D., Rauen T., Griffiths R., Sinclair C., Fumagalli E., Mennini T., *J. Med. Chem.*, **44**, 2507—2510 (2001).
 - 184) Eskandari S., Kreman M., Kavanaugh M. P., Wright E. M., Zampighi G. A., *Proc. Natl. Acad. Sci. U.S.A.*, **97**, 8641—8646 (2000).
 - 185) Wadiche J. I., Amara S. G., Kavanaugh M. P., *Neuron*, **15**, 721—728 (1995).
 - 186) Levy L. M., Warr O., Attwell D., *J. Neurosci.*, **18**, 9620—9628 (1998).
 - 187) Kanai Y., Trotti D., Nussberger G., Hediger M. A., “Neurotransmitter Transporters: Structure, Function and Regulation,” ed. by Reith M. A. E., Humana Press, Totowa, NJ, 1997, pp. 171—213.
 - 188) Slotboom D. J., Konings W. N., Lolkema J. S., *Microbiol. Mol. Biol. Rev.*, **63**, 293—307 (1999).
 - 189) Slotboom D. J., Konings W. N., Lolkema J. S., *FEBS Lett.*, **492**, 183—186 (2001).
 - 190) Grunewald M., Kanner B. I., *J. Biol. Chem.*, **275**, 9684—9689 (2000).
 - 191) Kanner B. I., Kavanaugh M. P., Bendahan A., *Biochem. Soc. Trans.*, **29**, 707—710 (2001).
 - 192) Seal R. P., Leighton B. H., Amara S. G., *Neuron*, **25**, 695—706 (2000).
 - 193) Vandenberg R. J., Arriza J. L., Amara S. G., Kavanaugh M. P., *J. Biol. Chem.*, **270**, 17668—17671 (1995).
 - 194) Kavanaugh M. P., Bendahan A., Zerangue N., Zhang Y. M., Kanner B. I., *J. Biol. Chem.*, **272**, 1703—1708 (1997).
 - 195) Zarbiv R., Grunewald M., Kavanaugh M. P., Kanner B. I., *J. Biol. Chem.*, **273**, 14231—14237 (1998).
 - 196) Zhang Y. M., Bendahan A., Zarbiv R., Kavanaugh M. P., Kanner B. I., *Proc. Natl. Acad. Sci. U.S.A.*, **95**, 751—755 (1998).
 - 197) Nagao S., Kwak S., Kanazawa I., *Neuroscience*, **78**, 929—933 (1997).
 - 198) Dehnes Y., Chaudhry F. A., Ullensvang K., Lehre K. P., Storm-Mathisen J., Danbolt N. C., *J. Neurosci.*, **18**, 3606—3619 (1998).
 - 199) Fairman W. A., Amara S. G., *Am. J. Physiol.*, **277**, F481—F486 (1999).
 - 200) Marie H., Attwell D., *J. Physiol. (London)*, **520**, 393—397 (1999).
 - 201) Lin C. O., Orlov I., Ruggiero A. M., Dykes-Hoberg M., Lee A., Jackson M., Rothstein J. D., *Nature (London)*, **410**, 84—88 (2001).
 - 202) Jackson M., Song W., Liu M.-Y., Jin L., Dykes-Hoberg M., Lin C. I., Bowers W. J., Fedoroff H. J., Sternweiss P. C., Rothstein J. D., *Nature (London)*, **410**, 89—93 (2001).
 - 203) Ginsberg S. D., Martin L. J., Rothstein J. D., *J. Neurochem.*, **65**, 2800—2803 (1995).
 - 204) Levy L. M., Lehre K. P., Walaas S. I., Storm-Mathisen J., Danbolt N. C., *Eur. J. Neurosci.*, **7**, 2036—2041 (1995).
 - 205) McGonigle P., Molinoff P. B., “Basic Neurochemistry, 4th edition,” ed. by Siegel G., Agranoff G. B., Albers R. W., Molinoff P. B., Raven Press, NY, U.S.A., 1989, pp. 183—202.
 - 206) Diamond J. S., Jahr C. E., *J. Neurosci.*, **17**, 4672—4687 (1997).
 - 207) Otis T. S., Jahr C. E., *J. Neurosci.*, **18**, 7099—7110 (1998).
 - 208) Grewer C., Watzke N., Wiessner M., Rauen T., *Proc. Natl. Acad. Sci. U.S.A.*, **97**, 9706—9711 (2000).
 - 209) Dredge B. K., Polydorides A. D., Darnell R. B., *Nature Rev. Neurosci.*, **2**, 43—50 (2001).
 - 210) Donly B. C., Jevnikar J., McLean H., Caveney S., *Insect. Biochem. Mol. Biol.*, **30**, 369—376 (2000).
 - 211) Gundersen V., Danbolt N. C., Ottersen O. P., Storm-Mathisen J., *Neuroscience*, **57**, 97—111 (1993).
 - 212) Gundersen V., Ottersen O. P., Storm-Mathisen J., *Eur. J. Neurosci.*, **8**, 758—765 (1996).
 - 213) Nakamura Y., Iga K., Shibata T., Shudo M., Kataoka K., *Glia*, **9**, 48—56 (1993).
 - 214) Nakamura Y., Kubo H., Kataoka K., *Neurochem. Res.*, **19**, 1145—1150 (1994).
 - 215) Nakamura Y., Kataoka K., *J. Mol. Neurosci.*, **4**, 255—262 (1994).
 - 216) Diamond J. C., Jahr C. E., *J. Neurophysiol.*, **83**, 2835—2843 (2000).
 - 217) Olie S. H., Piet R. R., Poulain D. A., *Science*, **292**, 923—926 (2001).