Excitatory amino acid research in Alzheimer’s disease: enhancement and blockade of receptor functions

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Introduction

The primary cause(s) of neurodegenerative diseases, including ischaemic and seizure-related brain damages, are far from fully elucidated [1]. Several factors may play important roles in the etiology of these diseases, including free radical formation and, perhaps, autoimmune mechanisms. Studies in recent years have, however, been focused on the role of the central excitatory amino acid (EAA) neurotransmitters, (S)-glutamic acid (Glu) and (S)-aspartic acid, in processes causing neuron injury and, ultimately, death [2]. The view that hyperactivity of central Glu neurons is an important causative factor in neurodegenerative processes (‘excitotoxicity’) is supported by in vitro and in vivo studies in model systems [2].

In the progression of Alzheimer’s disease, loss of Glu neurons is also observed [3]. The resulting hypoactivity of central EAA neuronal pathways has recently been proposed to be associated with the learning and memory deficits observed in Alzheimer patients [3, 4]. There is some evidence that hypoactivity of central EAA system(s) may also be a factor of importance in schizophrenia [5].

Since hyperactivity as well as hypoactivity of EAA neuronal systems may be implicated in Alzheimer’s disease, drugs capable of both protecting and activating EAA receptors may be of therapeutic interest. Partial agonists with appropriately balanced agonist/antagonist profiles [6, 7], or compounds capable of enhancing EAA receptor functions, may be of interest in Alzheimer’s disease as potential learning- and memory-stimulating drugs.

Excitatory amino acid receptor subtypes

Until a few years ago, EAAs were thought to mediate their actions through three different classes of receptors [8]. As a result of extensive neurochemical, pharmacological, and, in recent years, molecular biological studies, central EAA receptors are now most conveniently subdivided into five main classes, some of which, if not all, are heterogeneous [9, 10].

Class 1 are NMDA receptors at which N-methyl-D-aspartic acid (NMDA), quinolinic acid (QUIN) and ibotenic acid are agonists. NMDA receptors are competitively and very effectively blocked by a number of phosphonoamino acids, notably D-2-amino-5-phosphono pentanoic acid (D-AP5).

Class 2 are AMPA receptors at which quisqualic acid (QUIS) is a non-selective and (S)-2-amino-(3-hydroxy-5-methylisoxazol-4-yl)propanoic acid; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; EAA, excitatory amino acid; GDEE, glutamic acid diethyl ester; IP$_3$, inositol 1,4,5-trisphosphate; KA/N, kainic acid; NMDA, N-methyl-D-aspartic acid; QUIN, quinolinic acid; QUIS, quisqualic acid.
potent, but non-selective, antagonists at AMPA receptors [12]. More recently, (S)-2-amino-3-[3-carboxymethoxy]-5-methylisoxazol-4-yl]propanoic acid [(S)-AMOA] (see Figure 2) has been shown to antagonize AMPA-induced excitation, showing less effect on excitations by QUIS or kainic acid (KAIN) [13].

KAIN receptors, which are selectively activated by KAIN comprise the third class of EAA receptors. A number of other naturally occurring amino acids show very potent KAIN agonist actions [10]. So far, specific KAIN receptor antagonists are not available, but AMNH, which is structurally related to AMOA, selectively antagonizes excitation by KAIN [13].

The fourth class of EAA receptors consists of metabotropic receptors, which are coupled to inositol 1,4,5-trisphosphate (IP₃) and diacylglycerol turnover [14, 15]. At the metabotropic receptors, QUIS and ibotenic acid are non-selective agonists, the former compound being the most active agonist described so far. The trans form of 1-aminocyclopentane-1,3-dicarboxylic acid (trans-ACPD), notably the (1S,3R)-enantiomer, appears to be a selective agonist at the metabotropic receptor [15].

The final class consists of L-AP₄ receptors, through which L-2-amino-4-phosphonobutanoic acid (L-AP₄) inhibits synaptic excitation [15, 16].

**Specific excitatory amino acid receptor agonists derived from ibotenic acid**

Ibotenic acid is chemically unstable and, as an attempt to develop chemically stable compounds showing potent and specific NMDA effects, a number of ibotenic acid analogues structurally related to NMDA have been synthesized and tested, including (RS)-2-amino-2-(3-hydroxy-5-methylisoxazol-4-yl)acetic acid (AMAA) and related monocyclic and bicyclic analogues. None of these compounds showed detectable affinity for AMPA or high- and low-affinity KAIN binding sites, and AMAA was shown to be more potent than NMDA as an NMDA agonist [17].

As mentioned earlier, AMPA is a specific and very potent agonist at AMPA receptors, and radio-labelled AMPA has become the standard ligand for studies of this subtype of EAA receptors [18, 19]. A variety of structurally related 3-isoxazolol amino acids, including the very potent bicyclic homologue, (RS)-2-amino-3-(3-hydroxy-7,8-dihydro-6H-cyclohepta[1,2-d]isoxazol-4-yl)propanoic acid (4-AHCP), have been designed and tested in vivo and in vitro [20]. The tert-butyl analogue of AMPA, ATPA, is an agonist at AMPA receptors, albeit somewhat weaker than AMPA. Interestingly, replacement of the bulky and spherical tert-butyl group of ATPA by the planar phenyl group produces a compound, (RS)-2-amino-3-(3-hydroxy-5-phenylisoxazol-4-yl)propanoic acid (APPA), which shows markedly lower potency and efficacy than ATPA as an AMPA receptor agonist. APPA, actually, is the first example of a partial agonist at AMPA receptors [6].

**Stereostructure–activity studies on AMPA receptor agonists**

The stereochemical requirements for activation of AMPA receptors are being mapped out [10]. The
Figure 2

Structures of AMPA and AMOA, and the effects of (S)- and (R)-AMOA on currents induced by high (100 μM) and low (6 μM) AMPA concentrations (for details see [25])

(S)- and (R)-isomers of AMPA [21] and of Br-HIBO [22] have been synthesized using enzymic procedures. In both cases, the Glu diethyl ester (GDEE)- or CNQX-sensitive excitatory effects reside in the (S)-enantiomer, and there is a positive correlation between potency as neuroexcitants and affinity for AMPA receptor sites of the enantiomers of AMPA and Br-HIBO (Figure 1) [21,22].

However, whereas neither enantiomer of AMPA affects the binding of tritiated Glu in the presence of calcium chloride, (S)- and (R)-Br-HIBO are quite effective inhibitors in this binding assay, the latter enantiomer being the more potent inhibitor (Figure 1). The physiological relevance of this binding system is unclear, but these effects may somehow be associated with the enhancement of the excitatory effect of (S)-Br-HIBO by simultaneous electrophysiological application of (R)-Br-HIBO, which per se is inactive (Figure 3). Since (RS)-Br-HIBO is a more potent neuroexcitant than (S)-Br-HIBO, this enhancement does not simply reflect expression of a subthreshold excitatory effect of (R)-Br-HIBO in the presence of the (S)-enantiomer [22]. An even more pronounced enhancement of (R)-Br-HIBO of the excitation of neurons in the rat cortical wedge preparation by (S)-Br-HIBO has been demonstrated recently (U. Madsen, unpublished results).

The mechanism(s) underlying these enhancements are unknown. They may be mediated by as yet unknown pre- or post-synaptic transport or receptor mechanisms, respectively. There is evidence to suggest that the binding site for Glu in the presence of calcium chloride represents an uptake system different from synaptosomal or vesicular uptake [23].

These aspects, which may have pharmacological interest in relation to Alzheimer’s disease, are under further investigation.

AMOA: stereospecific antagonism and enhancement of excitation by AMPA

In the rat cortical wedge preparation, AMOA (Figure 2) was shown to antagonize, competitively, excitation induced by AMPA, with some selectivity [13]. On cat spinal neurons, AMOA reduced AMPA- and KAIN-induced excitations, whereas the excitatory effects of QUIS were less sensitive [13].

AMOA effectively protected rat striatal neurons against the neurotoxic effects of KAIN, whereas the toxic effects of AMPA were only slightly reduced. AMOA did not show protection...
against cell damage caused by intrastriatal injection of the NMDA agonist QUIN [13]. In cultured cortical neurons, AMOA effectively protected against KAIN-induced toxicity, whereas no significant protective effect could be demonstrated when the cells were exposed to AMPA or NMDA [24].

These comparative studies on AMOA as a non-NMDA receptor antagonist and as a neuroprotective agent strongly suggest that the mechanisms underlying neuroexcitation and neurotoxicity are not identical. Thus, it may be possible to develop neuroprotective agents acting at EAA receptors but showing little or no effect on EAA neuroexcitatory mechanisms.

It has been shown recently [25] that AMOA, at submillimolar concentrations, was capable of reducing KAIN-induced currents in mouse brain mRNA-injected *Xenopus* oocytes. The parallel shift to the right of the dose-response curve is consistent with AMOA acting as a competitive KAIN receptor antagonist [25] (not illustrated). Furthermore, because the reversal potential of the KAIN response was not modified, it may be concluded that the ionic selectivity of the KAIN-induced ion channel was not altered by AMOA [25]. In this study AMOA was found to have a dual action on AMPA receptors: (i) inhibition of responses induced by low concentrations of AMPA; and (ii) enhancement of responses by high concentrations of AMPA (Figure 2). The antagonist profile of AMOA is in agreement with the results of excitation studies in the cat spinal cord, where only relatively low concentrations of AMPA were used [13]. On the other hand, the enhancement by AMOA at high concentrations of AMPA (Figure 2) [25] may explain the lack of protective effect of AMOA on cortical neuronal cell death induced by high concentrations (100 μM) of AMPA [24]. A quantitative analysis showed that the apparent *K*<sub>d</sub> value of AMOA with respect to its potentiation of AMPA responses was about 25-fold lower than its *K*<sub>i</sub> value for inhibition of AMPA responses [25]. These results seem to indicate that potentiation and inhibition of AMPA-induced responses by AMOA are mediated by different sites. Importantly, both of these effects of AMOA were produced by the (S)-enantiomer of AMOA, whereas (R)-AMOA did not show significant effects on currents induced by AMPA (Figure 2) or KAIN [25].

The mechanism(s) underlying the enhancement of AMPA responses by AMOA are unknown. Direct single-channel experiments were required to determine how AMOA affects the function of the AMPA-gated ion channel.

Different mechanisms are probably involved in the enhancement of (S)-Br-HIBO-induced excitation by (R)-Br-HIBO (Figure 3), and the enhancement by AMOA of AMPA responses (Figure 2). However, both of these effects suggest that it may be possible to develop EAA-stimulating drugs, devoid of direct agonist effect. Such compounds may be of therapeutic interest in the treatment of Alzheimer's disease.

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Excitatory amino acid antagonists and epilepsy
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Exogenous excitatory amino acid (EAA) agonists (glutamate or aspartate) have convulsant action when administered focally into cortical or subcortical structures [1]. Likewise, more potent agonists acting selectively at the N-methyl-D-aspartate (NMDA) or at the a-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)/kainate (KA) receptors have both excitotoxic and proconvulsant/convulsant activity when administered systemically to rodents or primates (see [2-3]). Recently, a selective agonist acting at the metabotropic receptor, 1-amino-cyclopentane-trans-1,3-dicarboxylic acid (t-ACPD) has also been shown to induce convulsions in rats when administered focally in the hippocampus (see Schoepp, this colloquium). Conversely, antagonists acting at both NMDA receptors (competitive antagonists, non-competitive channel-site antagonists, non-competitive glycine site antagonists) and at non-NMDA (AMPA/KA) receptors have consistently exhibited anticonvulsant activity in a wide range of experimental seizure models (see [4]). Potent, selective antagonists for the metabotropic glutamate receptor are still lacking. Convulsions induced by t-ACPD can, however, be blocked by 1,2-amino-3-phosphonopropionic acid (AP3), known to act as an antagonist at the metabotropic receptor (see Schoepp, this colloquium).

The proconvulsant activity of EAA agonists and anticonvulsant activity of EAA antagonists is consistent with an up-regulated EAA transmitter system being one of the causative agents for enhanced seizure susceptibility in animal and human epilepsy. Numerous studies have been undertaken to establish a link between seizure susceptibility in animals or man and abnormalities in excitatory (or inhibitory) amino acid transmitter systems. On the whole, it has been difficult to correlate seizure susceptibility with any definitive alterations in endogenous, regional transmitter levels, turnover or release, or with abnormalities in regional excitatory and inhibitory receptor densities or affinities (see [3, 5, 6]). However, recent studies (conducted during and following surgery for refractory epilepsy), summarized in Table 1, point to a functional up-regulation of EAA transmission in brains of patients with partial (focal) seizures (temporal lobe epilepsy or epilepsy with frontal cortical foci) which resembles changes observed in certain chronic seizure models such as amygdala-kindled seizures. Whereas the altered hippocampal EAA binding pattern in chronic seizure models [7-11] or in human temporal lobectomy tissue [12-14] does not argue in favour of an overall up-regulation of the density of EAA receptors in epileptic tissue, there is accumulating evidence [15-18] of enhanced activity of NMDA or metabotropic receptors in limbic structures of chronically epileptic animals. The mechanism for such a functional upregulation (e.g. altered subunit composition, receptor phos-