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Glutamate release inhibitors: a critical assessment of their action mechanism

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Summary. A number of important experimental data do not support the widespread hypothesis that Na^+-channels block is cerebroprotective, essentially because it reduces presynaptic glutamate release: (i) the inhibition of exocytosis by these compounds is not specific to glutamate; (ii) aspartate efflux produced by various stimuli was also reduced, but aspartate cannot be released by exocytosis because it is not concentrated within presynaptic vesicles; and (iii) glutamate accumulated extracellularly during ischaemic or traumatic insult to the CNS is mainly of cytosolic origin. As an alternative, we propose that use-dependent Na^+-channel blockers enhance the resistance of nerve cells to insults, primarily by decreasing their energy demand, and that reduced efflux of glutamate and other compounds is a *consequence* of attenuated cellular stress.

Keywords: Glutamate release – Riluzole – Lamotrigine – Exocytosis – Excitotoxicity – Neuroprotection

Introduction

Excessive glutamate-mediated synaptic transmission may contribute to the pathophysiology of neurological disorders as varied as epilepsy, stroke, amyotrophic lateral sclerosis (ALS), and traumatic brain injury (Rothstein, 1996; Obrenovitch and Urenjak, 1997a). The potential clinical implications of this concept have prompted pharmaceutical companies to develop antagonists of postsynaptic NMDA- and AMPA/kainate-receptors. This notion has also encouraged claims that the neuroprotective effects of some drugs may result, *primarily*, from presynaptic inhibition of vesicular glutamate release (exocytosis). Those so-called "*(presynaptic) glutamate release inhibitors*" are use-dependent blockers of voltage gated Na^+-channels: e.g. lamotrigine, its derivatives BW1003C87 [5-(2,3,5-trichlorophenyl)-2,4-diaminopyrimidine ethane sulphonate] and BW619C89 [4-amino-2-(4-methyl-1-piperazinyl)-5-(2,3,5-trichlorophenyl pyrimidine], and riluzole (Urenjak and Obrenovitch, 1995).

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The purpose of this review is to assess objectively those claims, by addressing three important issues: (i) Are the so-called "*glutamate release inhibitors*" selective? (ii) Do these drugs block only the release of vesicular glutamate (i.e. exocytosis)? (iii) Is cerebroprotection with use-dependent Na^+-channel blockers linked to inhibition of glutamate exocytosis? Subsequently, we propose that down-modulation of voltage-gated Na^+-channels is potentially neuroprotective through a variety of mechanisms, among which inhibition of glutamate exocytosis is not the most important (Urenjak and Obrenovitch, 1998).

1. Are use-dependent blocker of voltage-gated Na^+-channels selective inhibitors of glutamate exocytosis?

As other classical neurotransmitters (e.g. dopamine, GABA, and acetylcholine), glutamate (*but not aspartate*) is stored at very high concentration (~100 mM) in presynaptic vesicles, of which a subpopulation is docked to the presynaptic plasmamembrane (Fig. 1). Under normal conditions, expulsion of glutamate from these vesicles (exocytosis) is triggered by action potential invasion of the presynaptic terminal, i.e. activation of voltage-dependent Na^+-channels. The resulting depolarization provokes an influx of Ca^{2+} through voltage-sensitive Ca^{2+}-channels (VSCC), ultimately leading to fusion of the vesicles with the presynaptic membrane and neurotransmitter release (Nicholls, 1993). Hence, exocytosis can be inhibited by Na^+-channel blockers (Cousin et al., 1993), Ca^{2+}-channel blockers (Pocock et al., 1995), or toxins which cleave proteins mediating the docking of vesicles with the presynaptic plasma membrane (Söllner and Rothman, 1994).

However, *selective* inhibition of glutamate exocytosis by acting on these molecular targets is inherently difficult because the processes depicted in

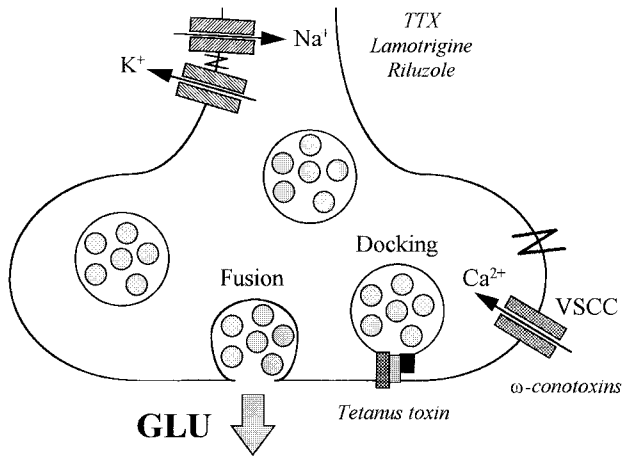

Fig. 1. Diagram of a presynaptic nerve terminal, illustrating key features of vesicular neurotransmitter release (i.e. exocytosis), and molecular targets for its inhibition: voltage-gated Na^+-channels, voltage-sensitive Ca^{2+}-channels *(VSCC)*, and vesicle docking. Drugs/toxin in italic are exocytosis inhibitors. *TTX* Tetrodotoxin

Fig. 1 are common to *all* neurotransmitters, even though the type(s) of Ca^{2+}-channel involved, and possibly the pattern of action potential burst required to trigger exocytosis may vary with the brain region and neurotransmitter under study (Nicholls, 1993; Pocock, et al., 1995). The fact that Na^+-channel blockers do not inhibit, selectively, glutamate exocytosis can be illustrated with lamotrigine (for riluzole, see Urenjak and Obrenovitch, 1997).

Lamotrigine concentration-dependently displaced BTX-B (batrachotoxinin A 20-a-benzoate, a selective ligand for the neurotoxin receptor site 2 of Na^+-channels) from rat brain synaptosomes (Cheung et al., 1992). Repetitive firing produced by depolarizing pulses in cultured neurons was blocked by lamotrigine in a concentration- (IC_{50} = 20 μM), voltage-, and use-dependent manner (i.e. lamotrigine had no effect on the first action potential elicited by a depolarizing step but reduced firing of subsequent action potentials) (Cheung et al., 1992). Increases in intracellular Na^+ or Ca^{2+} induced by the selective Na^+-channel activator, veratridine, were blocked by a series of reference Na^+-channel blockers, including lamotrigine (IC_{50} = 74–137 μM) (Deffois et al., 1996). Therefore, receptor-ligand data, functional tests and pharmacological studies demonstrate that lamotrigine is a use-dependent Na^+-channel blockers. As such, lamotrigine is a potential inhibitor of exocytosis, but this action is not restricted to the release of glutamate.

Lamotrigine inhibited veratridine-evoked glutamate release from rat cortical slices (IC_{50} = 21 μM), but it was only 2 times less potent in its inhibition of GABA release (IC_{50} = 44 μM) (Leach et al., 1991). This was substantiated by Waldemeier et al. (1995). In rat cortical or striatal slices, lamotrigine inhibited veratrine-induced release of endogenous glutamate, [^{3}H]-GABA and [^{3}H]-dopamine, with IC_{50} = 23, 40 and 45 μM in respective order, i.e. within the concentration range at which the drug interacts with Na^+-channels. Lamotrigine also inhibited the electrically induced release of [^{3}H]-noradrenaline, [^{3}H]-5-hydroxytryptamine and [^{3}H]-acetylcholine, although less potently than the release elicited by veratrine (Waldmeier et al., 1995). The lack of selectivity of lamotrigine inhibition of neurotransmitter release was confirmed by intracerebral microdialysis (Ahmad et al., 1995). In addition, Waldmeier et al. (1996) found that, at doses which totally suppress convulsions, lamotrigine caused no inhibition of veratridine-induced glutamate release in the brain striatum, and at best a 50% reduction in the cortex.

2. Which type of release did "*glutamate-release inhibitors*" block in some studies?

Two important features of the subcellular distribution of glutamate must be stressed: firstly, the pool of *vesicular* (or neuronal) glutamate (Fig. 2) is small, compared to that of *cytoplasmic* (or metabolic) glutamate (~10 mM in neurons and glia); and secondly, there is a 1 : 10,000 concentration gradient of glutamate across cellular membranes, because the extracellular concentration of glutamate is maintained at ~1 μM by acidic amino acid transporters (Nicholls, 1993). These processes, present in the membrane of both glia and neurons, are

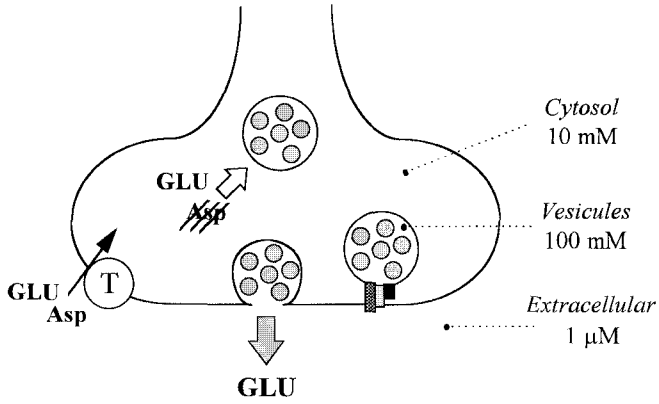

Fig. 2. Diagram of a presynaptic nerve terminal, illustrating the subcellular distribution of glutamate, and the fact that aspartate is transported across the cellular membrane by the high-affinity glutamate transporter, but NOT concentrated within synaptic vesicles. Accordingly, aspartate cannot be released by exocytosis and is not a neurotransmitter

electrogenic and tightly coupled to the transmembrane gradient of Na^+ and K^+ (Kanai et al., 1993). It is also established that pathological and experimental situations associated with altered transmembrane ion homeostasis (e.g. ischaemia, sustained K^+- or drug-induced depolarization) lead to increased extracellular concentrations of glutamate, which are primarily due to efflux of *metabolic* (i.e. cytosolic) glutamate (see next section). In these conditions, increased extracellular glutamate levels may reflect deficient uptake (Szatkowski et al., 1990; Zerangue and Kavanaugh, 1996) and/or cell swelling-induced efflux of glutamate, aspartate and other amino acids through a specific mechanism involving anion transport (Kimelberg et al., 1990).

Riluzole was reported to inhibit the efflux of aspartate provoked by various stimuli. At 50–100 μM, this drug inhibited the Ca^{2+}-dependent release of preloaded [^{3}H]-aspartate, produced by addition of 20 μM glutamate to the medium superfusing cultured cerebellar granule cells (Drejer et al., 1986; Hubert and Doble, 1989). Within the range 1 μM to 1 mM, riluzole concentration-dependently inhibited ibotenic acid-evoked release of [^{3}H]-aspartate (Hubert and Doble, 1989). At 10–30 μM, riluzole also reduced to a similar extent the release of glutamate and aspartate induced by exposure of rat hippocampal slices to 50 mM K^+ for 1 min (Martin et al., 1993). Similarly, lamotrigine attenuated the efflux of aspartate from rat brain cortex elicited by veratridine (Waldmeier et al., 1996). These data are generally interpreted as suggesting that "*glutamate release inhibitors*" also inhibit the exocytosis of aspartate. But, this endogenous excitatory amino acid is no longer considered as a neurotransmitter, because it is *not* concentrated into synaptic vesicles and, therefore, cannot be released by exocytosis (Fig. 2) (Nicholls, 1993; Orrego and Villanueva, 1993).

Efflux of cytosolic aspartate and glutamate may occur from three different mechanisms. When low concentrations of glutamate (or another amino acid

transportable by the high-affinity glutamate uptake) are applied to the preparation, aspartate and glutamate may be released by heteroexchange through the transporter (Griffiths et al., 1994). When cellular depolarization is sustained (i.e. high K^+, veratridine, high concentrations of glutamate agonists, energy deprivation), cytosolic efflux may reflect impairment of acidic amino acid uptake or opening of stretch-activated anion channels (see above).

Accordingly, we propose that reduced efflux of aspartate (and glutamate to a large extent) by use-dependent Na^+-channel blockers may actually reflect one or several of the following mechanisms: (i) reduction of heteroexchange subsequent to inhibition of neurotransmitter uptake – the latter was demonstrated for riluzole (Samuel et al., 1992); (ii) suppression of depolarization by direct interaction with the depolarizing agent – this is certainly the case with veratridine; (iii) inhibition of cell swelling-induced cytoplasmic efflux – riluzole and BW1003C87 blocked swelling-activated anion channels when glial cells were exposed to hypotonic media (Bausch and Roy, 1996); and (iv) reduction of the load on Na^+/K^+-ATPase, subsequent to reduced Na^+ influx through voltage-gated Na^+-channels, which helps the cells to cope with the depolarizing stimulus – in other terms, a larger part of Na^+/K^+-ATPase working potential is available to compensate for the increased permeability of the cellular membrane to ions (Urenjak and Obrenovitch, 1998).

3. Are cerebroprotective effects of use-dependent Na^+-channel blockers linked to inhibition of glutamate exocytosis?

As lamotrigine, BW1003C87, BW619C89 and riluzole consistently attenuate ischaemia-induced efflux of glutamate (and aspartate in some studies), it is often proposed that inhibition of glutamate exocytosis is the primary action underlying the cerebroprotective effects of these drugs (Meldrum et al., 1992; Graham et al., 1993; Lekieffre and Meldrum, 1993; Chen et al., 1995; Shuaib et al., 1995; Tsuchida et al., 1996; unpublished observations of Plotkine et al., cited by Doble, 1996; Bacher and Zornow, 1997). However, this hypothesis conflicts with two relevant features, in addition to the considerations detailed in the above sections.

(i) *In vitro* and *in vivo* data demonstrate that glutamate efflux is primarily of metabolic origin. A large part of glutamate released by synaptosomes during cyanide-induced anoxia was independent of Ca^{2+} (Sanchez-Prieto and Gonzalez, 1988) whereas exocytosis is Ca^{2+}-dependent (Fig. 1). Glutamate was released from cultured astrocytes exposed to hypoxic-hypoglycaemic conditions and the magnitude of this release was larger than that from neurons, even though astrocytes do not have any presynaptic terminals (Ogata et al., 1992). During severe ischaemia, exocytosis could not be sustained for more than a few minutes, because exocytosis requires ATP (Söllner and Rothman, 1994) and magnetic resonance spectroscopy showed total ATP depletion within 10 min of ischemia (Shimizu et al., 1993). By using microdialysis coupled to on-line analysis of glutamate, we established that only a minor component of ischaemia-induced glutamate efflux is of vesicular origin

(Wahl et al., 1994; Obrenovitch, 1996). Ischaemia produces equivalent efflux of glutamate and aspartate (Obrenovitch et al., 1993) but, aspartate cannot be released by exocytosis (see above).

(ii) Some of these compounds were protective even when administered after transient ischaemia (Pratt et al., 1992; Lekieffre and Meldrum, 1993), i.e. when extracellular glutamate levels had presumably returned to baseline (Ueda et al., 1992).

This rationale also applies to traumatic brain injury (Obrenovitch and Urenjak, 1997b). As an alternative mechanism, we propose that use-dependent Na^+-channel blockers enhance the resistance of nerve cells to insults, primarily by decreasing their energy demand, with reduced efflux of glutamate and other compounds being only a consequence of attenuated cellular stress (Urenjak and Obrenovitch, 1998).

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