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News and Commentary

Paradigm shift in neuroprotective drug development: clinically tolerated NMDA receptor inhibition by memantine

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Low-affinity, uncompetitive, open-channel blockers like memantine offer protection from dementia and other neurological disorders but break all the old rules of screening for new drugs by high-affinity binding. Here, in the occasion of this special issue dedicated to Bob Horvitz, we review four basic science concepts that are necessary to produce a clinically tolerated drug to treat human neurodegenerative disorders, including moderate-to-severe Alzheimer's disease.

Neurological Disorders are a Pervasive Problem

Studies in lower organisms, most notably the seminal work of Bob Horvitz in *Caenorhabditis elegans*, have contributed greatly to our understanding of cell injury and death mechanisms that also apply to human neurodegenerative disorders.[1] A number of genetic and pharmacological interventions have been shown to prevent this type of damage. Yet, treatment of the human condition is unique in that drugs that treat the brain must also not interfere with normal function. By definition, clinical neurology is an imperfect science because it 'takes the history of a diseased organ from a diseased organ.' Even when the proper location of disease is discovered in the nervous system and the diagnosis is correctly reached, the first rule of clinical intervention is that a drug must be safe and do no harm. And this has been a problem for many clinical studies of neuroprotective drugs.[2] Here, we recount novel insights into overcoming the problem of clinical tolerability and safety of effective neuroprotective drugs for the brain.

Acute and chronic neurological diseases are among the leading causes of death, disability, and economic expense in the world. As the population ages, care of patients with stroke and dementia is estimated to consume the entire US gross national product by the second half of this century. Hence, we are in dire need of new treatments. One final common mechanism that contributes to both acute and chronic neurodegenerative disorders is termed excitotoxicity (excessive activity of the neurotransmitter glutamate),[3] and this pathway has been implicated in neuronal injury and death due to either necrosis or apoptosis.[4] Neuronal injury often predominates in neurodegenerative disorders, with dendritic and synaptic damage as well as neuroinflammation,[5] all of which are potentially reversible if treated sufficiently early. With time, this damage can lead to frank neuronal cell loss. At present, we have no adequate treatments to prevent this damage or repair it. As an example, currently approved therapies for Alzheimer's disease slightly improve the symptoms of cognitive impairment by enhancing cholinergic function in the brain, but these drugs do not affect the injury and death pathways, and are thus not neuroprotective.

Excitotoxic damage is due in large measure to overstimulation of *N*-methyl-D-aspartate-type glutamate receptors (NMDARs) in the brain, with consequent excessive Ca^{2+} influx through the receptor's associated ion channel, contributing to detrimental enzymatic reactions, generation of toxic oxygen and nitrogen free radicals, and accumulation of abnormal protein aggregates. Hence, in an effort to prevent this type of damage, considerable effort has been put into developing pharmacological antagonists of the NMDAR. But one must remember that physiological NMDAR activity is essential for normal neuronal function, communication between neurons, and memory formation since glutamate is the major excitatory neurotransmitter in the brain.

The Problem with Conventional High-affinity Drugs

Most drugs are discovered by high-affinity screens for their target, in this case the NMDAR. Neuroprotective agents that work by high-affinity binding to the NMDAR end up blocking virtually all receptor activity; thus, these drugs manifest unacceptable clinical side effects, including hallucinations, drowsiness, and coma. For this reason, many NMDAR antagonists tested by big Pharma have disappointingly failed in advanced clinical trials.

In contrast to this high-affinity approach, we had proposed to protect the brain with drugs that do not bind very well under physiological conditions but are nevertheless selective under pathological conditions for a particular target, such as the NMDAR.[6–8] This proposal was met with great skepticism by many scientists and clinicians alike.

The First Concept: Mechanism of Open-channel Block and Uncompetitive Inhibition

The first important concept presented in this proposal concerning mechanism of drug action is that one type of

clinically tolerated yet neuroprotective NMDAR antagonist would be an 'open-channel blocker,' meaning that the drug enters the receptor-associated ion channel only when it is open. Importantly, this type of drug will be most effective in the face of excessive (pathological) activity because statistically more channels are open and available to be blocked. This mechanism of inhibition, whose action is contingent upon prior activation of the receptor by the agonist, is also termed 'uncompetitive' antagonism.

At a given concentration, an uncompetitive antagonist is more effective against excessive activity than physiological activity. In contrast, a conventional 'competitive' antagonist works better against physiological activity than pathological activity. Competitive antagonists compete one-for-one with agonist, thereby blocking healthy areas of the brain (where, on average, lower physiological activity exists) more than pathological areas (where there is excessive NMDAR activity). Drugs that simply compete for the agonist binding sites of the NMDAR (there are two, one for glutamate and another for glycine; see Figure 1), preferentially block normal function and therefore are not clinically tolerated, and have thus failed in trials because of side effects.

In fact, an uncompetitive open-channel blocker would prevent more severe excitotoxic processes better than mild disease. One would predict, for example, that moderate-to-severe dementia, involving excessive NMDAR activity leading to neuronal injury or death, would be treated more effectively than mild dementia or other excitotoxic disorders, involving only somewhat increased physiological firing. (Of course, these drugs will not reverse very severe disease because the neurons will already be lost.) Although preferential neuroprotection from moderate-to-severe excitotoxic processes seems counterintuitive, the drug's uncompetitive mechanism of action readily explains this uncanny phenomenon.

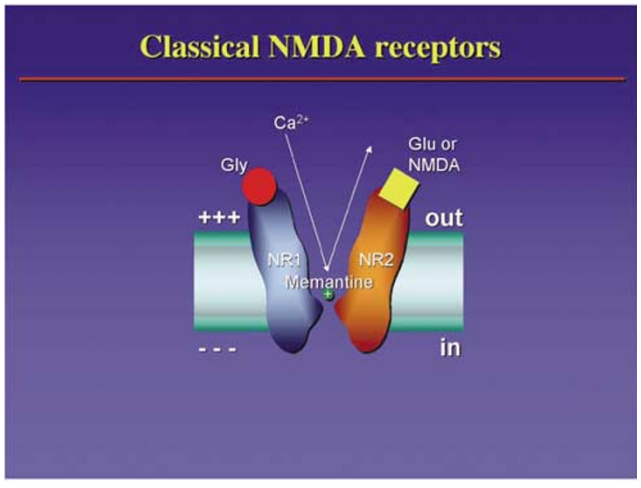

Figure 1 Open-channel blockers such as memantine inhibit the NMDAR preferentially when it is excessively (pathologically) activated but have little if any effect on normal synaptic transmission. (Glu=glutamate; Gly=glycine; NR1 and NR2 are subunits of the NMDAR that is probably composed of a tetramer of subunits)

The Second Concept: 'Off-rate' from the NMDAR-associated Ion Channel

Most importantly, we proposed that the real secret to designing such a clinically tolerated NMDAR open-channel blocker involves its kinetics of action within the ion channel.[6–9] Thus, the second important concept presented here concerns the drug's 'off-rate' from the channel. A relatively fast off-rate (and hence short dwell time in the channel) would prevent the drug from accumulating in open channels. This avoids progressive blockade of normal synaptic transmission.[10] In contrast, a drug with a slow off-rate would build-up in the ion channels that underlie synaptic events and consequently interfere with normal neurological function. The apparent affinity of a channel-blocking drug is related to its off-rate divided by its on-rate. At a given membrane potential, the on-rate is not only a property of diffusion and channel open probability, but also the drug's concentration. In contrast, the off-rate is an intrinsic property of the drug–receptor complex, unaffected by drug concentration. A relatively fast off-rate is a major contributor to a drug's low affinity for the channel pore. Thus, we propose that a clinically tolerated neuroprotective drug would consist of a low-affinity, open-channel blocker with a relatively fast off-rate. Hence, the drug would not substantially interfere with normal synaptic neurotransmission in an accumulative fashion. As a result, the drug will be both effective and well tolerated.

Drugs that Act like the 'Volume Control' on your Television Set *Versus* the 'On–off' Switch

To further highlight the mechanism needed for a safe yet effective drug, the NMDAR can be thought of as a television set. The agonist sites are like the 'on/off' switch of the television. Drugs that block here cut off all normal NMDAR function. What we need to find is the equivalent of the 'volume' control (or in biophysical terms, the gain) of the receptor. Then, excessive Ca^{2+} influx through the NMDAR-associated ion channel would be prevented by simply turning down the 'volume' of the Ca^{2+} flux toward normal values. A blocker that binds at a site within the channel, similar to the action of physiological levels of Mg^{2+}, could act as a sensor and provide an 'automatic' volume control. Importantly, the automatic volume control needs to reach an optimal level. In the case of Mg^{2+} itself, the block is too ephemeral, a so-called 'flickery block,' and the cell continues to depolarize (become positively charged because of Ca^{2+} and Na^{+} entry) until Mg^{2+} is repelled, and the block is totally relieved. Hence, in most cases Mg^{2+} does not effectively block excessive Ca^{2+} influx to the degree needed to prevent neurotoxicity. If, on the other hand, a channel blocker binds with too high an affinity, it will accumulate in the channels, block normal activation, and thus prove clinically unacceptable. Following the television set analogy, turning the volume all the way down is as bad as turning off the 'on/off' switch in terms of normal functioning of the television. This is the case with MK-801; it is a very good blocker of excitotoxicity, but because its dwell time in the ion channel is so long (reflecting its slow off-rate and high affinity),

it progressively blocks critical normal functions. MK-801 thus produces coma. Drugs with slightly shorter but still excessive dwell times (off-rates) make patients hallucinate (e.g., phencyclidine, also known as Angel Dust), or so drowsy that they classify as anesthetics (e.g., ketamine).

A clinically tolerated NMDAR antagonist would not make a patient drowsy, hallucinate, or comatose, and in fact should spare normal neurotransmission while blocking the ravages of excessive NMDA receptor activation. An uncompetitive, open-channel mechanism of blockade coupled with a longer dwell time in the channel (and consequently a slower off-rate) than Mg^{2+} but a substantially shorter dwell time (faster off-rate) than MK–801 would yield a drug that blocks NMDAR-operated channels only when they are excessively open, while relatively sparing normal neurotransmission.

The Third Concept: Targeting Additional Protective Agents to Sick Neurons via a Neuron-selective Drug

The third concept in drug design presented here concerns the ability of an uncompetitive antagonist to target other moieties to sick neurons. An NMDAR open-channel blocker binds increasing well to neurons manifesting excessive channel activity; these are by definition potentially vulnerable neurons. Hence, we can attach other antiapoptotic or prosurvival moieties to an open-channel blocker to form a new adduct that is targeted to vulnerable neurons, giving the second moiety specificity of action to the sick neuron. Using this concept, we propose that a series of second-generation drugs can be formulated that will have even greater neuroprotective properties than the original. These second-generation drugs can take advantage of the fact that the NMDAR has other modulatory sites (or 'volume' controls) in addition to its ion channel that offer safe but effective clinical intervention.

One example of an additional modulatory site(s) on the NMDAR that we can take therapeutic advantage of involves the action of nitric oxide (NO). We have shown that transfer of NO to thiol (−SH) groups on critical cysteine residues of the NMDAR (a reaction we and our colleagues have termed S-nitrosylation) decreases excessive receptor activity.[11–13] However, if administered systemically, NO can cause serious side effects, including severe hypotension (low blood pressure) by virtue of its ability to produce vasodilation, and may even be toxic by reacting with superoxide anion (O_2^-) to form peroxynitrite ($ONOO^-$). To avoid this problem, we can tether the NO group to an appropriate open-channel blocker in order to specifically target NO to the NMDAR nitrosylation sites. To date, such combinatorial drugs, linking the principles of open-channel block and S-nitrosylation of the NMDAR to provide two 'volume controls,' show great clinical potential.

The Fourth Concept: Pathologically Activated Therapeutics (PAT Drugs)

In the fourth and final concept in drug discovery presented here, we propose that this newly recognized mode of action for drugs be designated '*P*athologically *A*ctivated *T*herapeutics' or PAT (a gentle tap). By virtue of their relatively gentle binding, PAT drugs work best under pathological conditions, while exerting minimal effects on normal brain activity. We believe that these simple concepts embody the future of clinically tolerated neuroprotective drug design. Interestingly, we were the first to show that the adamantane derivative, memantine, fulfills these mechanistic criteria.[6–9] We showed that memantine was not only neuroprotective; but also an open-channel, uncompetitive antagonist of the NMDAR-associated ion channel with a relatively fast 'off-rate,' thus avoiding accumulation in the channels. Moreover, we showed that memantine does not substantially affect normal synaptic activity but prevents excessive NMDAR activity. Unlike other NMDAR antagonists that are currently available, side effects are thus averted with memantine because neurotransmission is preserved. This discovery led to several advanced clinical trials run by our group and by a number of colleagues.[14,15] As a result, the European Union approved memantine for the treatment of Alzheimer's disease last year. Recently, memantine also passed two US phase III clinical trials for moderate-to-severe Alzheimer's disease and was voted unanimous approval by an FDA Advisory Panel, thus representing the first neuroprotective drug to gain acceptance in the USA. Additional clinical studies of memantine for other forms of dementia, depression, and glaucoma are currently underway, and more effective second-generation drugs are sure to follow. In summary, basic science discoveries can lead to neuroprotective drugs in the clinic. However, a detailed understanding of the mechanism of neuronal cell injury and drug action is critical for this process to be successful.

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