Small Molecules Targeting the NMDA Receptor Complex as Drugs for Neuropathic Pain

Rosa Planells-Cases[1], Enrique Pérez-Payá[2], Angel Messeguer[2], Cristina Carreño[3] and Antonio Ferrer-Montiel[1,*]

[1]*Instituto de Biología Molecular y Celular, Universidad Miguel Hernández, Alicante, Spain;* [2]*Dpt. of Biological Organic Chemistry, IIQAB-CSIC, Barcelona, Spain;* [3]*DiverDrugs, SL, L´Hospitalet de Llobregat, Barcelona, Spain*

Abstract: Pain is a complex disease that usually remains poorly treated or undertreated, especially the neuropathic pain caused by injury to the peripheral or central nervous system. Antagonists of the NMDA receptor complex have emerged as potential drugs for pain management. A strong case is being raised for non-competitive or uncompetitive antagonists with low-to-moderate affinity and fast on/offset kinetics as drugs with good therapeutic profiles, because of their reduced side effects.

Keywords: Excitatory activity, nerve damage, inflammation, ion channels, protein networks, uncompetitive antagonists, synaptic plasticity, drug discovery, pain killers.

INTRODUCTION

Pain is an unpleasant sensation primarily designed to avoid tissue damage. Pain is considered a disease that requires aggressive physician treatment. Remarkably, the number of patients suffering pain has been increasing up to half a billion cases per year. In both its acute and chronic forms, pain has a major impact on many quality-of-life measures [1]. For example, chronic pain sufferers account for 25% of the adult population, of which approximately 5% experience pain that is poorly treated and can be debilitating, resulting in loss of work, family crisis, depression and/or suicide [2,3]. Moreover, it is estimated that at least 50% of individuals seeking treatment are unsatisfied with their present pain management options. Thus, the economic and medical costs of inadequate pain therapy in the community are obviously enormous.

The molecular mechanisms involved in pain transduction have remained largely elusive due, in part, to the subjective nature of pain sensation. Recent advances in the molecular biology, genetics and physiology of sensory transduction are starting to unfold the components and organization of the signalling pathways involved in pain transduction. This remarkable progress will be undoubtedly translated into better pain management strategies.

Although pain has been traditionally considered as a unique and homogeneous pathology, continuing research advances have established that pain is an extremely complex and dynamic process involving multiple, interrelated neurotransmitter/neuromodulator systems in the peripheral nervous system. Indeed, as many as 15 neurotransmitters have been implicated in diverse aspects of pain-processing pathways [4-6]. Two major types of pain are widely recognized, namely inflammatory and neuropathic. Inflammatory pain is produced in response to tissue damage [4,7]. Inflammatory pain involves various painful responses resulting from peripheral tissue injury and/or inflammation produced by trauma, infection, surgery, burns or diseases with an inflammatory component. In most cases, inflammatory pain is mediated by pro-algesic mediators such as protons, histamine, cytokines, prostaglandins, kinins, chemokines and ATP [4,7]. These molecules sensitise neurons in the pain pathway (known as nociceptive neurons or nociceptors), in the majority of cases by directly modulating the sensitivity of membrane receptors or up-regulating intracellular signalling cascades. As a result, strong sensory signalling is conveyed to the spinal cord and subsequently to specific brain regions leading to pain sensation. In addition, central sensitization at the level of the spinal cord further augments the pain sensation [4,6]. Depending on the duration, this sort of pain is classified as acute or chronic.

Neuropathic (or neurogenic) pain is elicited by injury to the peripheral or central nervous system due to tissue trauma, infection or autoimmune diseases such as nerve degeneration in diabetes and postherpetic neuralgia (shingles), and various cytostatic drugs [8]. Neuropathic pain does not appear related with nociceptor sensitization and is thought to arise from over-activation of ion channels on postsynaptic neurons in the gray matter of the spinal cord [8]. Microarray analysis of the neuronal transcriptome in an animal model of neuropathic pain reveals a complex molecular profile, primarily characterized by the expression of genes involved in neuronal regeneration [9]. No significant changes in the RNA levels of ion channels are detected. Pharmacology-based findings using ion channel antagonists, however, reveal a predominant role of this kind of membrane proteins, especially of ionotropic glutamate receptors (iGluRs). Of the three types of iGluRs, NMDA receptors are intimately involved in signalling neuropathic pain [10-13]. There is growing evidence that hyperalgesia and allodynia are largely mediated by excessive excitation of NMDA receptors [12,13]. Prolonged activation of this receptor is thought to reorganise pain-sensing circuits and develop the hypersensitivity of neuropathic pain. Because of

*Address correspondence to this author at the Instituto de Biología Molecular y Celular, Universidad Miguel Hernández, Av. Ferrocarril s/n, 03202 Elche (Alicante), Spain; Tel: +34 966658727; Fax +34 966658758; E-mail aferrer@umh.es

1389-5575/03 $41.00+.00 © 2003 Bentham Science Publishers Ltd.

their implication in this intractable sort of pain, NMDA receptors have emerged as key therapeutic targets for neuropathic pain management.

PHYSIOLOGY AND PATHOLOGY OF NMDA RECEPTORS

Glutamate is the main excitatory neurotransmitter in the mammalian brain. Three types of glutamate-gated channels transduce the synaptic signal: NMDA, AMPA, and kainate receptors. NMDA receptors are abundant in excitatory postsynaptic membranes, although some are reported presynaptic, ubiquitously distributed throughout the nervous system and critical for the normal function [14]. NMDA receptors are known to play important physiological roles such as cognition, brain development and synaptic plasticity associated with memory formation, as well as several pathological states. Indeed, NMDA receptors have been involved in acute neurodegeneration (e.g. stroke), in chronic degeneration (Alzheimer´s, Huntington´s and Parkinson´s diseases) and in processes such as chronic pain [10-13].

The NMDA receptor is a heteromeric channel composed of an NR1 subunit in combination with one or more NR2 subunits (NR2A to D) and, occasionally, an NR3 (NR3A-B) subunit. There is controversy whether the stoichiometry of the receptor is pentameric or tetrameric [10]. Further receptor diversity is achieved by alternative splicing of NR1 subunits to yield eight alternative forms in the amino- and carboxi-terminal domains. The four NR2 subunits are generated by four distinct genes that share between 50-70% homology. NR1 and NR2A are ubiquitous, NR2B is most abundant in forebrain structures, NR2C in cerebellum and NR2D is modestly expressed and restricted to subcortical areas. NR2A and 2B appear to co-localize in different brain structures such as in cortex and thalamus, whereas NR2D appears with NR1, NR2A and NR2B subunits in the rat thalamus. Specific assembly seems regionally and developmentally regulated which, in part, could be determined by interaction with specific different splice-variants of NR1 [15]. At birth, NMDAR from forebrain are mostly composed of NR2B which are substituted during postnatal development by NR2A [10,14].

Activation of NMDA receptors requires binding of both glutamate and glycine to open the channel pore and to allow cation entry, mainly the calcium. The channel is open in a voltage-dependent manner since it is blocked by magnesium at resting membrane potentials, whereas the magnesium blockade is released at depolarized potentials. The receptor activity is modulated by the redox state and by low concentrations of polyamines [10]. Furthermore, the NMDA receptor is the target for the anti-convulsant dizolcipine (MK-801), the dissociative agent ketamine and the drug of abuse phencyclidine (PCP or angel dust). These drugs produce psychotomimetic symptoms, such as hallucinations, which may be related to complete abrogation of NMDA receptor activity [13].

The NMDA receptor is a complex membrane protein with a variety of potential sites for drug targeting. These sites include the ligand-binding site, a strychnine-insensitive glycine ($glycine_B$) site, a polyamine site at the pore vestibule, and a PCP site inside the channel pore. Opportunities for modulating the receptor activity are further increased by the molecular diversity of receptor subunits and splice forms that result in heteromeric assemblies with distinct biophysical and pharmacological properties. Numerous structure-function studies are defining the molecular determinants of these receptor-binding sites with the aim of providing a blueprint of the channel structure that could be of use for rational drug design. These studies have revealed that the agonist-binding site is located in the NR2 subunits, while the $glycine_B$ site is structured in the NR1 subunit [10].

Pharmacological, genetic and behavioural studies have provided evidence for the role of NMDAR in pain. In spinalized rats, but not in intact animals, NMDA is able to induce long-term potentiation in C-fibres, a cellular effect that may be important in central hyperalgesia [16]. This enhancement could provide an underlying mechanism for persistent pain. In neuropathic pain, in which there is an excessive activity of excitatory pathways, NMDA receptor blockers have been reported to be analgesics [17].

Mice lacking NR1 and each of the NR2 subunits have been generated. Mice deficient for NR1 ($NR1^{-/-}$) die perinatally and show a concomitant reduction of NR2B but not of NR2A [18,19]. A mouse model that expresses reduced (5-10%) levels of NR1 was characterized and displayed hyperlocomotion, increased stereotypy and abnormal social behaviour [20]. $NR2A^{-/-}$, $NR2C^{-/-}$ and $NR2D^{-/-}$ mice have been generated that show deficiencies either in spontaneous activity or learning abilities. Unfortunately, no effect on nociception has been reported for these mouse models yet [21-25].

Recently, a null mutant of PSD-95, a multivalent-adaptor protein that interacts with NMDA receptors in the dorsal-horn of the spinal cord, has been reported that fails to develop NMDA receptor-dependent hyperalgesia and allodynia. In contrast, $PSD\text{-}95^{-/-}$ mice display normal inflammatory nociceptive behaviour [26]. Calcium Calmodulin-kinase CaMKinase associates in a PSD-95 dependent manner with the NMDA receptor after nerve injury. Damage-induced re-organization of functional domains containing the NMDA receptor may also be critical for pain sensitization.

Among all subunits, the NR2B subunit has been implicated in pain perception. Mice that overexpress NR2B in forebrain, specifically in anterior cingulate and insular cortices, exhibit enhanced responsiveness to hindpaw injection of inflammatory stimuli (formalin injection). No differences in tail-flick response or in the latency to react to noxious temperature stimuli were, however, observed [27]. Forebrain regions, such as the anterior cingulate cortex, are important in pain perception and plastic changes are observed after tissue injury or amputation. NR2B was overexpressed in rostral ventromedial medulla (RVM) after complete Freund´s adjuvant injection [28]. This increase lasted until 2 weeks after inflammation. A concomitant increment of on- and off-like cells was observed that suggests a change in excitability in the RVM pain modulatory circuitry [28]. Currently, a case for the expression of the NR2B in the peripheral nervous system is building up [29-31,15]. However, the specific role of peripheral NMDA receptors in pain transmission is still

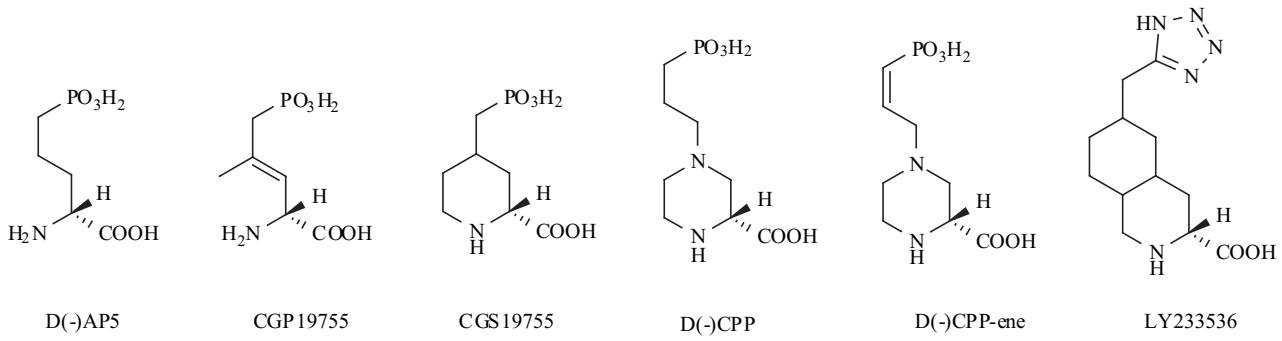

Fig. (1). Competitive NMDA antagonists.

elusive, since most of the effects appear mediated by those expressed in the forebrain [29]. Collectively, these findings indicate that NMDA receptors, primarily those containing NR2B subunit, are central players in pain transduction and, thus, validated drug targets for novel analgesics [32].

PHARMACOLOGY OF THE NMDA RECEPTORS

The NMDA receptor is a complex molecular entity with various drug-binding sites. This property has prompted the discovery of different kinds of receptor modulators with the hope that they will be of clinical use to treat NMDA receptor dysfunction. However, because of the relevant role of this excitatory receptor in the physiology of the CNS, soon was realized that the full abrogation of channel activity that may lead to serious unwanted side effects. As a result, drug discovery platforms and medicinal chemists began to focus on strategies to reduce the side effect concerns raised by NMDA receptor antagonists. The most significant secondary effects elicited by this sort of drugs include psychotomimetic actions, cognitive disruption, euphoria reward and addiction, and neurotoxicity. Exciting advances in this field are highlighted next.

COMPETITIVE ANTAGONISTS

Because NMDA receptors are ligand-gated ion channels, the pharmacological and medicinal efforts were focussed on developing compounds that bind to the L-glutamate site (Fig (**1**)). Analysis of structure-activity relationships (SAR) provided a blueprint for the topology of this important receptor site [33]. Mutagenesis studies identified key amino acid residues involved in ligand binding [14]. Together, these findings were used to further refine the design of competitive NMDA antagonists. As a result, more specific and potent compounds were designed and tested *in vitro* and *in vivo*. However, indiscriminate blockade of NMDA receptors was deleterious for a number of physiological processes. Most of the clinical trials with these compounds had to be halted because of the side effects concerns such as sedation, confusion, muscular weakness and ataxia [34]. Nonetheless, a take home message that emerged from these findings was the need to design drugs that clearly distinguish the population of pathologically working receptors from that mediating physiological synaptic transmission.

NON-COMPETITIVE ANTAGONISTS

Compounds that do not recognize the L-glutamate binding site and bind to the NMDA receptor in a voltage-independent manner are known as non-competitive antagonists. Two major sites are considered: a) the glycine$_B$ site; and, b) the channel vestibule.

THE GLYCINE$_B$ SITE

The glycine$_B$ site on the NMDA receptor has been considered a target for drug intervention [35]. Glycine is an allosteric effector of the receptor which modulates the rate and extent of desensitization. Notably, glycine specifically acts on NMDA receptor, with no interaction with the other subtypes of ionotropic glutamate receptors. Thus, competitive glycine antagonists were developed with the trust they should improve receptor selectivity (Fig. (**2**)). Among these, moderate partial antagonists, such as D-serine, D-cycloserine and HA-966, or full agonists, such as kynurenic and 7-chloro-kynurenic, were assayed. These compounds selectively blocked NMDA responses with micromolar affinity [35]. They barely interacted with other ionotropic glutamate receptors, although these compounds did not distinguish between the different types of heteromeric NMDA receptors. *In vivo* experiments with the kynurinate family were quite disappointing because its members exhibited very poor brain bioavailability which was translated in weak activity when given systemically [36].

At variance, the partial glycine agonist HA-966 displayed higher systemic bioavailability. Interestingly, the glycine$_B$ antagonism was specifically restricted to the (R)-(+)-enantiomer, while the (S)-(-)-enantiomer contained the γ-butyrolactone-like sedative effects [37]. In an attempt to increase the compound affinity, the (R)-(+)-cis-β-methyl-HA-966 (L-687,414) was obtained. This derivative exhibited a 10-fold increased affinity for the glycine$_B$ site [37]. Similarly, improvement in the systemic bioavailability of the molecule was obtained. *In vivo*, compound L-687,414 exhibited neuroprotective and anticonvulsant activity, although still displayed sedative/ataxic effects at therapeutic relevant doses [37].

A third class of glycine$_B$ antagonists is represented by the tricyclic pyrido-phthalzine-dione derivatives developed by researchers at Merz and Co [38] (Fig. (**2**)). These

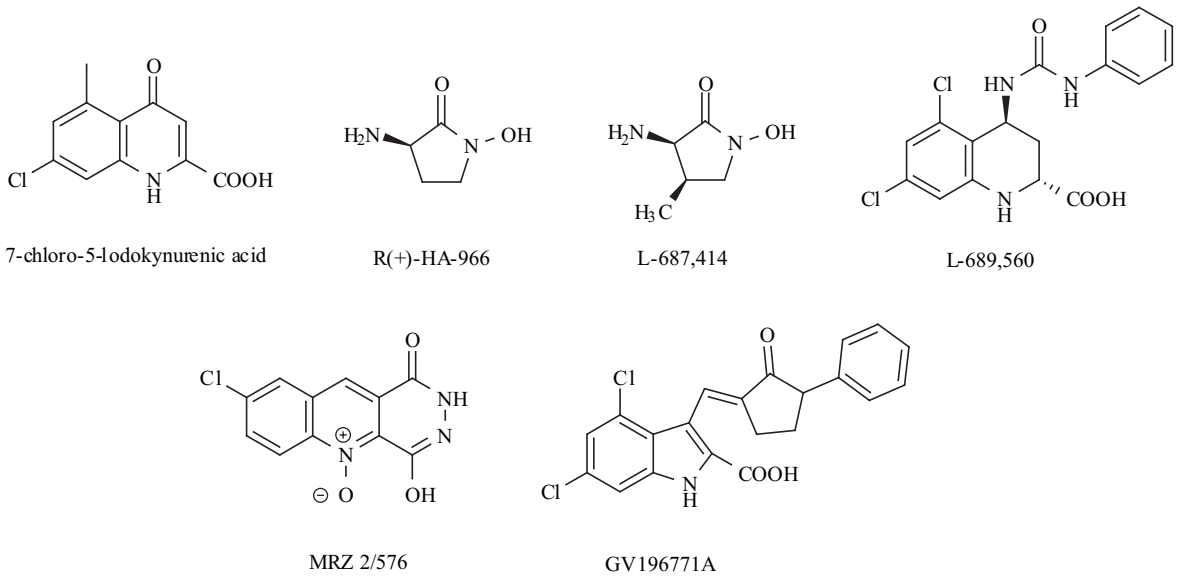

Fig. (2). Glycine$_B$ site antagonists.

compounds blocked NMDA receptor channel activity with moderate-to-low activity (50-0.1 μM) and were designed with the aim to improve the systemic bioavailability of glycine$_B$ antagonists. The mechanism of action indicated that they act as full glycine antagonists. Notably, at variance with other glycine$_B$ antagonists, tricyclic pyrido-phthalzine-diones blocked more potently the plateau NMDA currents than the peak NMDA currents. This property, along with their moderate affinity, appears important since it may efficiently expose NMDA receptor glycine-sensitive desensitization which, in turn, may underlie a better therapeutic profile [17]. Indeed, receptor desensitization may represent a safety mechanism to reduce the activity of the receptor and prevent its long-term activation that ultimately is neurotoxic. Hence, compounds that allow the initial receptor response but rapidly ensure its desensitization should not attenuate significantly synaptic activity, but notably repress prolonged activation of the receptor. The family of tricyclic pyrido-phthalzine-dione derivatives may fulfil these expectations by providing NMDA receptor inhibitors with lower side effects.

THE CHANNEL VESTIBULE

Positively charged residues, such as dynorphins, have been shown to inhibit NMDA receptor activity non-competitively (Fig (**3**)). These naturally occurring opioid peptides are found in the spinal cord and caudal medulla where they reduce the firing frequency of dorsal neurons and lengthen the latency of tail flick reflexes evoked by noxious stimuli. Levels of these opiod peptides augment markedly upon spinal cord trauma. Dynorphins act directly on κ-opioid receptors and NMDA receptors. Their glutamatergic action is voltage-independent and appears to be sensitive to the redox state of the receptor [39]. These peptides have several positively charged residues that critically contribute to their antagonistic activity. In addition, amidation of the carboxylate end increases 10-fold their affinity for the NMDA receptor. The high content of positive charges and the higher activity of the amidated form are consistent with an interaction with negatively charged residues located at the pore entryway [40]. A plausible binding site would be that occupied by Ca^{2+} ions. In support of this tenet, poly-arginine hexapeptides identified by combinatorial chemistry strongly blocked NMDA receptor activity by recognizing a binding site in the pore vestibule [41].

Dynorphin A	GGFLRRIRPKLKWDNQKRYGGFLRRQFKVVT-NH_2
Arginine-rich peptide	RRRRWW-NH_2
Conantokin T	GEEEYQKMLENLREAEVKKNA-NHX_2
Conantokin G	GEEELQENQELIREKSN-NH_2
Conantokin R	GEEEVAKMAAELARENIAKGCKVNCYP-NH_2

Fig. (3). Non-competitive NMDA antagonist.

In vivo, dynorphins exhibited antinociceptive activity, consistent with both their interaction with κ-opioid receptors and NMDA receptors. Paradoxically, these opioid peptides are centrally involved in the induction of persistent nociception characterized by long-lasting mechanical, tactile and cold allodynia [42]. An emerging tenet for the dual *in vivo* activity of dynorphins invokes that the analgesic activity is mediated by its interaction with opioid receptors, while binding to the NMDA receptor is responsible for its pro-algesic activity [43]. The discovery that NMDA receptor-evoked responses are potentiated by dynorphin at low extracellular glycine concentrations indicates an excitatory activity that may underlie the provoked *in vivo* neuropathic pain and neurotoxicity [42,44]. Noteworthy, dynorphin-induced excitotoxicity is fully abrogated by NMDA receptor blockers, but insensitive to opioid receptor antagonists further substantiating the involvement of the NMDA receptor.

Conantokins are polypeptide toxins isolated from the *Conus* marine snails that selectively inhibit NMDA receptor activity (Fig. (**3**)) [45]. These toxins are non-competitive NMDA antagonists that appear to allosterically modulate the polyamine-binding site on the receptor, although the specifics of their binding site are elusive. Several subtypes

(G, L,T and R) of conantokins which differ in their blockade efficacy and receptor selectivity have been isolated and characterized. For instance, conantokin T blocks NR2A or NR2B subtype receptors, while conantokin G acts more efficiently on NR2B receptors [46]. Intrathecal administration of both peptides suppressed the ongoing pain behaviour in the formalin test at doses that were 17-27 times lower than those impairing motor activity [47]. Intriguingly, only conantokin G was effective attenuating thermal hyperalgesia and mechanical allodynia upon nerve injury [47]. The lack of analgesic activity of conantokin T was assigned to the absence of NR2A subtype of receptors in the spinal cord. However, a recent report has provided compelling evidence of the presence of NR2A subunits in the spinal cord [26]. Hence, further experiments seem necessary to clarify this discrepancy. Nonetheless, the marked analgesic activity of these toxins warrants further exploration aimed at developing them as useful analgesics for pain management, especially neuropathic pain. Clearly, efforts have to be made to unveil the structural determinants of their receptor-binding site as a first step to develop non-peptidic analogs of these toxins.

UNCOMPETITIVE ANTAGONISTS

Uncompetitive antagonists are a class of activity-dependent inhibitors that specifically bind to the agonist-receptor complex. Because of their interaction with active receptors, these compounds have attracted a notable interest for drug development. This property is considered as a solid argument for their preferential blockade of highly activated receptors and minimal interaction with physiologically working channels. High affinity uncompetitive NMDA antagonists include MK-801 and PCP that blocked the channel activity with low nanomolar activity (Fig. (**4**)). These blockers are potent neuroprotectants *in vitro* and *in vivo*. MK-801 also displays pronounced anti-nociception *in vivo*. Therapeutic activity of high-affinity uncompetitive antagonists is accompanied with psychotomimetic and ataxic secondary effects that prevent their clinical use. The marked adverse effects were attributed to the significant use-dependence of their blockade activity that results in a continuous and almost irreversible blockade of all receptors [36]. Use-dependence is primarily due to blocker trapping in its binding site upon agonist dissociation from the receptor. Blocker trapping is associated with a slow dissociation constant of the blocker from its binding site [48]. Hence, the use of antagonists that bind with moderate-to-low affinity and fast association/dissociation kinetics should bestow upon improved therapeutic profiles.

A relevant step in this goal was the characterization of low-affinity compounds such as ketamine, dextromethorphan, dextrorphan, memantine, ARL15896AR and NPS1506 (Fig. (**4**)) [17]. Ketamine and dextromethorphan are used clinically for the treatment of pain conditions, and are being clinically tested for additional pain states. Similarly, memantine has been approved for the treatment of Alzheimer and is being investigated for pain management. Other compounds are in clinical development as neuroprotectants and analgesics.

Recently, a novel class of low-affinity, highly specific uncompetitive antagonists were identified from the screening

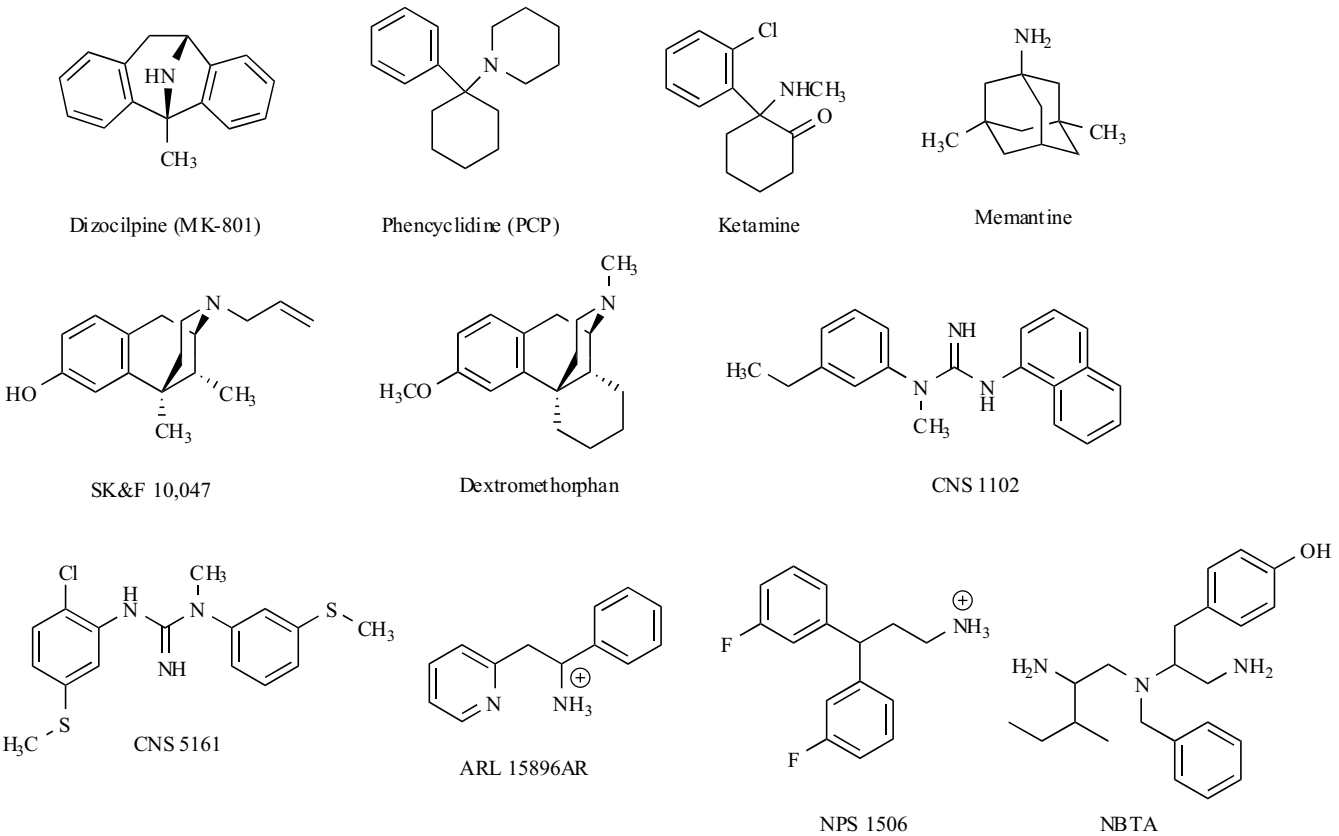

Fig. (4). Uncompetitive NMDA antagonists.

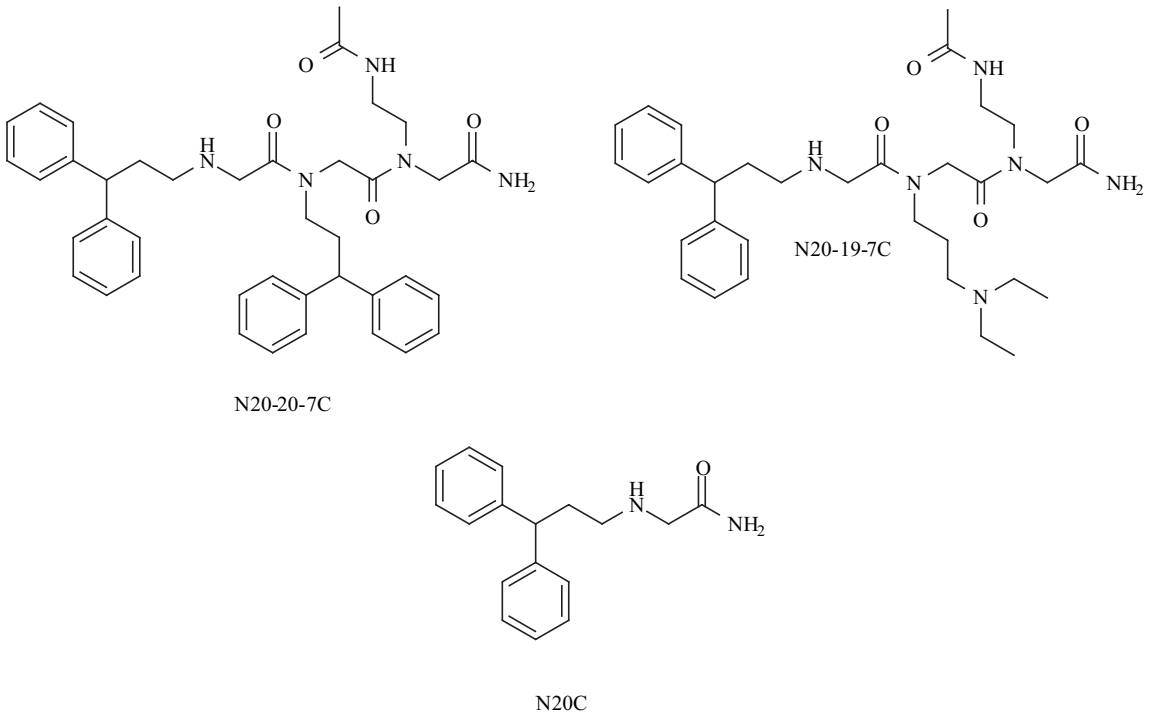

Fig. (5). N-alkylglycines blockers of the NMDA receptor.

of peptidomimetic-based libraries [49,50]. Peptidomimetic molecules such as *N*-alkylated glycines (also known as peptoids) constitute a family of non-natural compounds that exhibit interesting biological properties [51,52]. Formally, peptoids result from the shift of the substituent present at α-carbon atom in amino acids to the adjacent nitrogen atom (Fig. (**5**)). Nevertheless, although being isomers of peptides, peptoids show different structural features, such as the higher degree of conformational freedom and the absence of CO---NH hydrogen bonds. These properties modify the steric interactions leading to the secondary structure present in peptides. Moreover, contrary to what occurs in peptides, peptoid backbones are achiral and protease resistant. Peptidomimetic-based libraries were designed to circumvent the toxic *in vivo* activity exhibited by arginine-rich hexapeptides [41]. Screening of a library of 10,648 N-alkylated glycines identified a family of peptoids that selectively block the NMDA receptor channel activity with micromolar efficacy. Noteworthy, step-wise size reduction of the original trimers of *N*-alkylglycines identified compound N20C (3,3-diphenylpropyl-*N*-glycinamide), a low molecular weight, highly selective NMDA receptor that abrogated channel activity by an uncompetitive mechanism [50]. A similar blockade profile was shown for the *N*-benzylated triamine denoted as NBTA (Fig. (**4**)) [49]. Notably, N20C exhibited neuroprotective activity *in vitro* and *in vivo*. The *in vivo* activity was devoid of apparent motor and cognitive deficits, substantiating its therapeutic potential. Interestingly, N20C exhibited structural analogies with the NPS 1506 compound. Ongoing experiments in neuropathic pain models will prove its clinical usefulness.

Taking together, low-to-moderate affinity, fast on/offset kinetics and moderate voltage-dependence uncompetitive NMDA antagonists are compounds with a proven potential to discern between normal and pathological activation of NMDA receptors; this differentiation depends on the underlying channel activity characteristic of these conditions. Hence, these compounds efficiently undermine the low frequency activation of the NMDA receptor characteristic of pathological states such as wind-up, while leaving untouched the high frequency-stimulation that underlies long-term potentiation. New developments in this exciting field will provide compounds with much improved therapeutic profiles. A note of caution should be sound, however, because of a potential neurodegenerative activity associated with chronic use of these compounds [53,54]. This shortcoming may be circumvented by the discovery and development of subunit-specific uncompetitive antagonists.

RECEPTOR SUBTYPE-SPECIFIC ANTAGONISTS

Antagonists that selectively act on subtype-specific NMDA receptors are considered a promising approach to improve the efficacy and therapeutic profile of NMDA receptor blockers. Specifically, a remarkable effort is being devoted to discover antagonists of NR2B-containing NMDA receptors. NR2B subunits are primarily expressed in forebrain [13,29]. Overexpression of this subunit in these brain regions markedly enhances inflammatory pain preception [29]. Furthermore, NR2B protein was detected in the spinal cord and primary sensory neurons, suggesting an involvement in pain transmission [31, 55]. However, a role of the NR2A subunit in pain must not be excluded since expression of this receptor subunit was also discovered in the spinal cord [26].

Pharmacologically, attenuation of NR2B-containing receptors has resulted in pain-relief. Ifenprodil, the first NR2B-specific antagonist discovered, and related compounds have been found to be antinociceptive in a

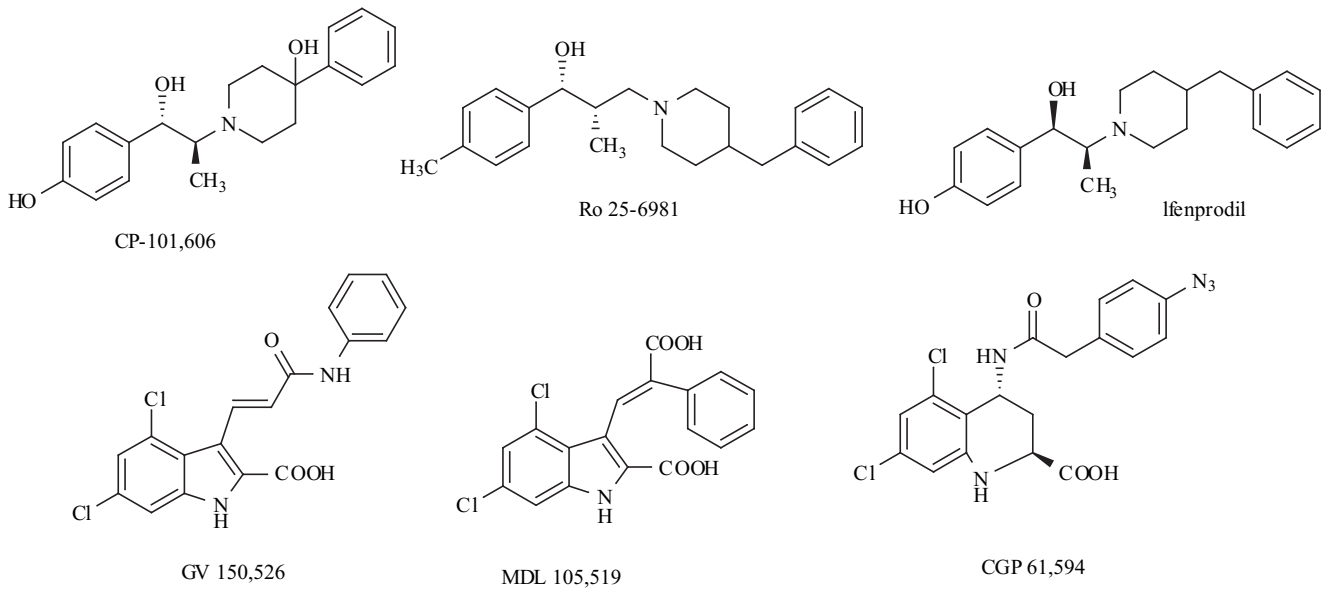

Fig. (6). NR2B-subtype receptors selective inhibitors.

variety of preclinical pain models (Fig. (**6**)). Most significantly, these compounds are active in attenuating neuropathic pain at doses devoid of anticonvulsive, cognitive and ataxic side effects [30]. These compounds inhibit NMDA receptor channel activity by non-competitive, voltage-independent mechanism. These compounds bind to the N-terminus domain of the NR2B subunit, allosterically interacting with the proton sensor and the polyamine-binding site [56,57]. The initial expectations for these compounds suffered a serious set back when it was discovered that they exhibit high affinities for α_1-adrenoreceptors and other ion channels [30]. Moreover, several of the NR2B selective agents also interacted with human ether-a-go-go (HERG) K^+ channels, which result in the prolongation of the Q/T interval and serious cardiac problems [17]. SAR analysis has partly differentiated between the structural determinants of the NR2B antagonism and those required acting on other channels. This achievement turned out in the design of CP-101,606, Ro 25-69981, GV150,526 and MDL 105,519 which exhibit lower receptor crossreactivity [30] (Fig. (**6**)). Nonetheless, the development of more selective NR2B antagonists, devoid of cardiac side effects, remains an open challenge. In addition, their pain-relief activity on humans trials awaits confirmation.

MODULATORS OF THE NMDA RECEPTOR SIGNALLING COMPLEX

Novel sites for drug intervention in the NMDA receptor multiprotein complexes (also known as the postsynaptic signalling complex) have been uncovered by extensive proteomic analysis of synaptic terminals [58]. These complexes are composed of a plethora of proteins that include receptor, adaptor, signalling, cytoskeletal and novel proteins. This signalling complex is organized by the PSD-95 protein, a multivalent adaptor protein that mediates protein-protein interactions through PDZ domains. Assigned functions to the postsynaptic signalling complex are the distribution and clustering of NMDA receptors and their co-localization with signalling proteins for an efficient transduction of external signals. Thus the postsynaptic protein complex contains a variety of drug targets. Apart from the enzymes that participate in signalling, it appears that disruption of the protein network may be of therapeutic interest because of the uncoupling of NMDA receptor channel activity and intracellular pathways. In support of this tenet, it was recently proven that perturbing NMDA receptor-PSD95 protein interactions with small peptides has an impressive neuroprotective effect *in vivo* [59]. Furthermore, disruption of the NMDA receptor-signalling complex in the spinal cord eliminated the NMDA receptor-mediated hyperalgesia and allodynia in a model of neuropathic pain [26]. Together, these findings highlight the potential of modulating the integrity of protein complexes as new strategies for pain management. It should be mentioned that the discovery and development of small molecules that modulate protein-protein interactions remains an unmet goal of current drug discovery.

PERSPECTIVES

The unquestionable involvement of the NMDA receptor in pain transduction has thrust a renowned interest on the development of antagonists of this neuronal receptor for pain management. The NMDA receptor offers multiple sites for drug targeting. This is apparently an easy task for current drug discovery platforms, although the real challenge is to develop antagonists that preserve the physiological activity of these subtypes of ionotropic glutamate receptors while correcting over-active receptors. Therefore, physiological constraints have to be included in the development and optimization of new NMDA receptor antagonists to ensure their clinical utility as pain killers.

ACKNOWLEDGMENTS

The authors thank all colleagues and members of their research and collaboration groups for their fundamental

contribution to the results presented herein . Financial support grants from MCYT, Fundació La Caxia, Fundació Marató TV3, and FIS are acknowledged.

REFERENCES

[1] Russo, C.M.; Brose, W.G. *Annu. Rev*., **1998**, *49*, 123.
[2] Wolf, C.; Salter, M.W. *Science,* **2000,** *288*, 1765.
[3] Brower, V. *Nat. Biotech*., **2000**, *18,* 387.
[4] Williams, M.;Kowaluk, E.A.; Arneric, S.P. *J. Med. Chem.*, **1999**, *42*, 1481.
[5] McCleskey, E.W.; Gold, M.S. *Annu. Rev. Physiol*., **1999,** *61*, 835.
[6] Cesare, P.; McNaughton, P. *Curr. Opin. Neurobiol*., **1997**, *7*, 493.
[7] Wood, J.N.; Docherthy, R. *Annu. Rev. Physiol*., **1997**, *59*, 457.
[8] Zimmermann, M. *Eur. J. Pharmacol.,* **2001**, 429,23.
[9] Xiao, H.-S.; Huang, Q.-H.; Zhang, F.-X.; Bao, L.; Lu, Y.-J.; Guo, C.;Yang, L.; Huang, W.-J.; Fu, G.; Xu, S.-H.; Cheng, X.-P.; Yan, Q.; Zhu, Z.-D.; Zhang, X.; Chen, Z.; Han, Z.-G.; Zhang, X. *Proc. Natl. Acad. Sci. USA,* **2002** *99*, 8360.
[10] Dingledine, R.; Borges, K.; Bowie, D.; Traynelis, S.F. *Pharmacol. Rev.,* **1999**, *51*, 7.
[11] Eide; K., Stubhang; A., Oye, I.; Breivik, H. *Pain*, **1995**, *61*, 221.
[12] Eisenberg, E.; Pud, D. *Pain*, **1998**, *74*, 337.
[13] Felsby, S.; Nielsen, J.; Arandt-Nielsen, L.; Jensen, T.S. *Pain*, **1996**, *64*, 283.
[14] Collingridge, G.L.; Watkins, T.C. *The NMDA receptor*, Oxford University Press: Oxford, **1994**.
[15] Loftis, J.M.; Janowski, A. *Pharmacol. Ther.,* **2003,** *97*, 55.
[16] Liu, X.-G.; Sandkühler, J. *Neurosc*., **1998,** *86*, 1209.
[17] Parsons, C.G. *Eur. J. Pharmacol.,* **2001**, *429*, 71.
[18] Forrest, D.; Yuzaki, M.; Soares, H.D.; Ng, L.; Luk, D.C.; Sheng, M., Stewart, C.L.; Morgan, J.I.; Connor, J.A.; Curran, T. *Neuron*, **1994**, *13*, 325.
[19] Li, Y.; Erzurumlu, R.S.; Chen, C.; Jhaveri, S.; Tonegawa, S. *Cell,* **1994** *76*, 427.
[20] Mohn, A.R. ; Gainetdinov, R.R.; Caron, M.G.; Koller, B.H. *Cell,* **1999***, 98*, 427.
[21] Ikeda, K.; Araki, K.; Takayama, C.; Inoue, Y.; Yagi, T.; Aizawa, S.; Mishina, M. *Brain Res. Mol. Brain Res.*, **1995,** *33*, 61.
[22] Kutsuwada, T.; Sakimura, K.; Manabe, T.; Takayama, C.; Katakura, N.; Kushiya, E.; Natsume, R.; Watanabe, M.; Inoue, Y.; Yagi, T. *et al. Neuron,* **1996,** *16*, 333.
[23] Sakimura, K.; Kutsuwada, T.; Ito, I.; Manabe, T.; Takayama, C.; Kushiya, E.; Yagi, T.; Aizawa, S.; Inoue, Y.; Sugiyama, H.; *et al. Nature,* **1995,** *373*, 151.
[24] Ebralidze, A.K.; Rossi, D.J.; Tonegawa, S.; Slater, N.T. *J. Neurosci,* **1996,** *16,* 5014.
[25] Das, S.; Sasaki, Y.F.; Rothe, T.; Prekumar, Y.F.; Tasaku, M.; Crandall, J.E.; Dikkes, P.; Conner, D.A.; Rayudu, P.V.; Cheung, W.; *et al. Nature,* **1998,** *393*, 377.
[26] Garry, E.M.; Moss, A.; Delaney, A.; O´Neill, F. Blakemore, J.; Bowen, J.; Husi, H.; Mitchell, R.; Grant, S.G.N.; Fleetwood-Walker, S.M. *Curr. Biol.,* **2003***,13*, 321.
[27] Wei, F.; Wang, G.-D.; Kerchner, G.A.; Kim, S.J.; Xu, H.-M.; Chen, Z.-F.; Zhuo, M. *Nature Neuroscience,* **2001,** *4*, 164.
[28] Miki, K.; Zhou, Q.-Q.; Guan, W; Guan, Y.; Terayama, R.; Dubner, R.; Ren, K. *J. Neurophysiol*., **2002**, *87*, 750.
[29] Zhuo, M. *Drug Discov.Today*, **2002**, *7*, 259.
[30] Chizh, B.A.; Headley, P.M.; Tzschentke, T.M. *Trends in Pharmacol. Sci.,* **2001,** *22,* 636.
[31] Boyce, S.; Wyatt, A.; Webb, J.K.; O´Donnell, R.; Mason, G.; Rigby, M.; Sirinathsinghji , D.; Hill, R.G.; Rupniak, N.M.J. *Neuropharmacol.,* **1999,** *38*, 611.
[32] Kemp, J.A.; McKernan, R.M. *Nature neuroscience*, **2002**, *5*, 1039.
[33] Jane, D.E.; Olverman, H.I.; Watkins, J.C., In *the NMDA Receptor*, **1994**, Collingridge, G.L.; Watkins, T.C. *The NMDA receptor*, Oxford University Press: Oxford, **1994,** pp. 31.
[34] Meldrum, B.S.; Chapman, A.G. In *the NMDA Receptor,* **1994**, Collingridge, G.L.; Watkins, T.C. *The NMDA receptor*, Oxford University Press: Oxford, **1994,** pp. 457.
[35] Danysz, W.; Parsons, A.C. *Pharmacol. Rev.,* **1998,** *50*, 597.
[36] Iversen, L.L.; Kemp, J.A. In *the NMDA Receptor*, Collingridge, G.L.; Watkins, T.C. *The NMDA receptor*, Oxford University Press: Oxford, **1994,** pp. 467.
[37] Singh, L.; Donald, A.E.; Foster, A.C.; Hutson, P.H.; Iversen, L.L.; Kemp, J.A.; Leeson, P.D. Marshall, G.R.; Priestley, T.; Thorn, L.; Tricklesbank, M.D.; Vass, C.A.; Williams, B.J. *Proc. Natl. Acad. Sci. USA,* **1990**, *87*, 347.
[38] Parsons, C.G.; Danysz, W.; Quack, G.; Hartmann, S.; Lorenz, B.; Wollenburg, C.; Baran, L.; Przegalinski, E.; Kostowski, W.; Krzascik, P.; Chizh, B.; Headley, P.M. *J. Pharmacol. Exp. Ther.,* **1997,** *283*, 1264.
[39] Chen, L.; Huang, L.-Y. M. *J. Pharmacol. Exp. Ther.*, **1998**, *284*, 826.
[40] Hauser, K.F.; Knapp, P.E.; Turbek, C.S. *Exp. Neurol.,* **2001***, 168*, 78.
[41] Ferrer-Montiel, A.V.; Merino, J.M.; Blondelle, S.E.; Pérez-Payá, E.; Houghten, R.A.; Montal, M. *Nature biotechnology*, **1998**, *16*, 286.
[42] Laughlin, T.M.; Larson, A.A.; Wilcox, G.L. *J. Pharmacol. Exp. Ther.,* **2001***, 299,* 6.
[43] Tan-No, K.; Esashi, A.; Nakagawasai, O.; Niijima, F.; Tadano, T.; Sakurada, C.; Sakurada, T.; Bakalkin, G.; Terenius, L.; Kisara, K. *Brain Res.,* **2002***, 952*, 7.
[44] Zhan, R.Z.; Fujiwara, N.; Yamakura, T.; Taga, K.; Fukuda, S.; Endoh, H.; Shimoji, K. *Brain Res.*, **1997**, *760*, 179.
[45] Castellino, F.J.; Prorok, M. *Curr. Drug. Targets,* **2000,** *1*, 219.
[46] Klein, R.C.; Warder, S.E.; Galdzicki, Z.; Castellino, F.J.; Prorok, M. *Neuropharmacol.,* **2001***, 41*, 801.
[47] Malmberg, A.B.; Gilbert, H.; McCabe, R.T.; Basbaum, A.I. *Pain,* **2003,** *101,* 109.
[48] Mealing, G.A.; Lanthorn, T.H.; Small, D.L.; Murray, R.J.; Mattes, K.C.; Comas, T.M.; Morley, P. *J. Pharmacol. Exp. Ther.,* **2001**, *297*, 906.
[49] Tai, K.-K.; Blondelle, S.; Ostresh,J.M.; Houghten, R.A.; Montal, M. *Proc. Natl. Acad. Sci. USA***, 2001,** *98*, 3519.
[50] Planells-Cases, R.; Montoliu, C.; Humet, C.; Fernández, A.; García-Martínez, C.; Valera, E.; Merino, J.M.; Pérez-Payá, E.; Messeguer, A.; Felipo, V.; Ferrer-Montiel, A. *J. Pharmacol. Exp. Ther.,* **2002,** *302*, 163.
[51] Miller, S. M.; Simon, R. J.; Ng, S.; Zuckermann, R. N.; Kerr, J. M.; Moss, W. H. *Bioorg. and Med. Chem. Lett.,* **1994**, *4*, 2657.
[52] Simon, R. J.; Kania, R. S.; Zuckermann, R. N.; Verena, D.; Huebner, V. D.; Jewell, D. A.; Banville, S.; Ng, S.; Wang, L.; Rosenberg, S.; Marlowe, C. K.; Spellmeyer, D. C.; Tan, R.; Frankel, A. D.; Santi, D. V.; Cohen, F. E.; Bartlett, P. A. *Prod. Natl. Acad. Sci. USA,* **1992**, *89*, 9367.
[53] Ikonomidou, C.; Bosch, F.; Miksa, M.; Bittigan, P.; Vockler, J.; Dikranian, K.; Tenkova, T.I.; Stefovska, V.; Turski, L.; Olney, J.W. *Science*, **1999**, *283*, 70-74.
[54] Ikonomidou, C.; Stefovska, V.; Turski, L. *Proc. Natal. Acad. Sci. USA*, **2000**, *97*, 12850.
[55] Ma, Q.P.; Hargreaves, R.J. *Neuroscience,* **2000,** *101,* 699.
[56] Kew, J.N.; Kemp, J.A. *J. Physiol.,* **1998**, *512*, 17.
[57] Mott, D.D.; Doherty, J.J.; Zhang, S.; Washburn, M.S.; Fendley, M.J., Lyuboslavsky, P.; Traynelis, S.F.; Dingledine, R. *Nat. Neurosci.,* **1998**,*1*, 659.
[58] Husi, H.; Ward, M.A.; Choudhary, J.S.; Blackstock, W.P.; Grant, S.G.N. *Nature neuroscience*, **2000**, *3*, 661.
[59] Aarts, A.; iu, Y.; Liu, L.; Besshoh, S.; Arundine, M.; Gurd, J.W.; Wang, Y.-T.; Salter, M.W.; Tymianski, M. *Science***, 2002**, *298*, 846.

Copyright © 2003 EBSCO Publishing