

# Metabotropic Glutamate Receptor Involvement in Models of Acute and Persistent Pain: Prospects for the Development of Novel Analgesics

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**Abstract:** The excitatory amino acid glutamate plays a major role in nociceptive processing. Ionotropic and metabotropic glutamate receptors are expressed in relevant areas of the brain, spinal cord and periphery that are involved in pain sensation and transmission. Activation of mGlu receptors along the pain neuraxis can result in either pronociceptive or antinociceptive behaviors depending on the subtype of mGluR and its location. The data published to date most strongly support the idea that mGlu1 antagonists might act as broad-spectrum analgesics. Several studies pointing to a functional upregulation of mGlu2/3 in chronic pain models suggest that agonists of these receptors might also be effective analgesics in certain conditions, most notably inflammation-induced hyperalgesia and allodynia. The expression of mGluRs throughout the pain neuraxis and the differing roles of the mGluRs in each of these regions makes it difficult to predict the efficacy of mGluR ligands based on *in vitro* or local administration studies. Potent, systemically active compounds that show mGluR subtype selectivity will be critical to undertake more detailed analyses in animal models of pain.

**Keywords:** Pain, allodynia, sensory neuron, antinociception, metabotropic glutamate receptors

## INTRODUCTION

Pathologic pain is a common, intractable problem confronting patients and their physicians. While physiologic, acute pain warns against incipient bodily harm, pathologic pain following tissue or nerve injury often persists after the injury has resolved and can be extremely debilitating. Tissue injury commonly results in persistently increased pain sensation to mildly noxious stimuli, known as *hyperalgesia*, and pain sensation from non-noxious stimuli, known as *allodynia*.

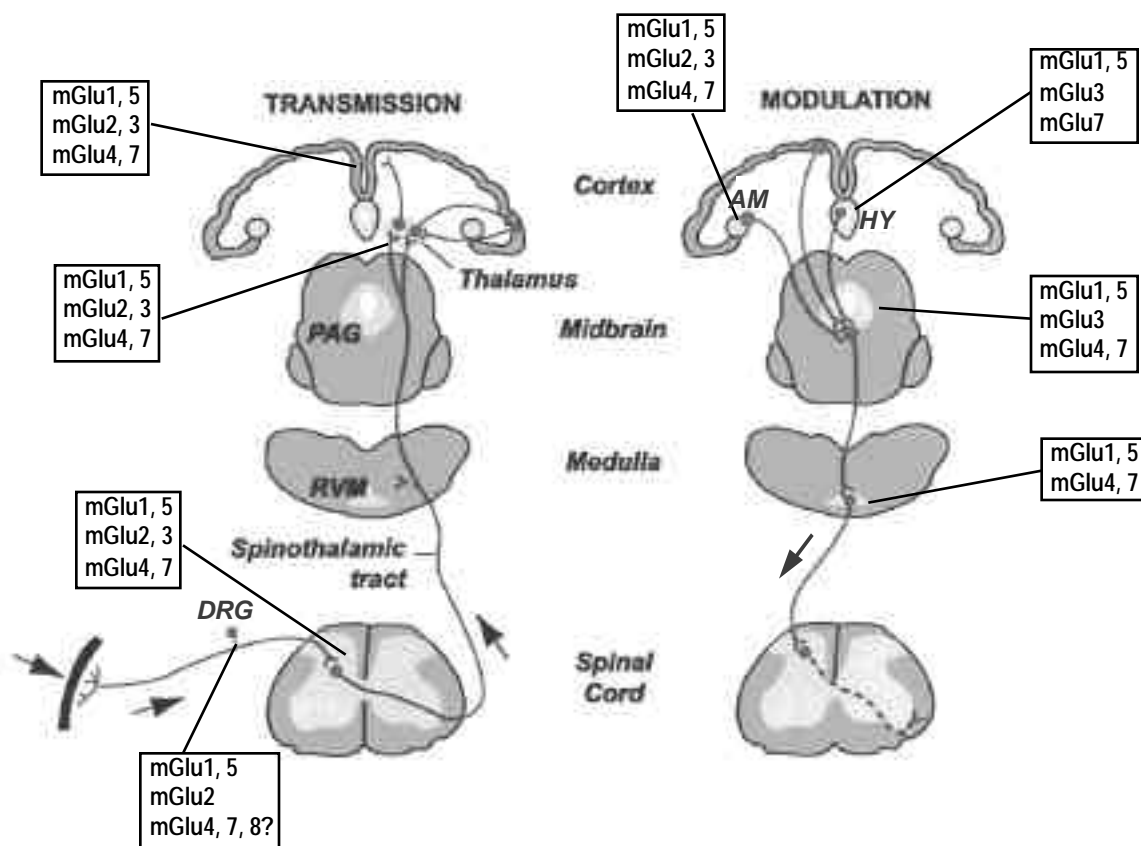
Injury and diseases of the peripheral nervous system can also result in chronic pain conditions, referred to as *neuropathic pain* [1]. Neuropathic pain is associated with severe, chronic sensory disturbances characterized by spontaneous pain, hyperalgesia and allodynia. Prevalent symptoms in human patients include cold hyperalgesia, mechanical allodynia, and less commonly, heat hyperalgesia [1, 2]. Approximately 4 million people in North America suffer from chronic neuropathic pain, and of these, it is estimated that no more than half achieves adequate pain control [3]. Moreover, neuropathic pain is often refractory to most commonly used analgesics, including opioids in humans [4-6] and rats [7, 8].

A cellular and molecular understanding of hyperalgesia and allodynia is essential to understanding and identifying

targets for the pharmacologic or genetic treatment of persistent pain. The transmission of nociceptive signals from the periphery to the spinal cord occurs through sensory neurons whose cell bodies lie in dorsal root ganglia (Fig. [1]). Noxious stimuli to the periphery activate receptors on nociceptive sensory neurons, causing them to fire and release glutamate and neuromodulatory peptides from the axon terminals in the spinal cord. The sensory neuron terminals form synaptic contacts with neurons in the dorsal horn of the spinal cord, and these cells project to sensory centers in the brain. Glutamate released from the sensory neuron terminal exerts its actions on the dorsal horn neurons via activation of two major classes of receptors: ionotropic glutamate receptors (iGluRs) and metabotropic glutamate receptors (mGluRs). The iGluRs are ligand-gated ion channels including NMDA, AMPA, and kainate receptors. These channels mediate fast synaptic transmission from sensory neurons onto dorsal horn neurons and in major sensory processing centers in the brain. The mGluRs comprise a family of receptors coupled to various intracellular second messenger systems through G proteins, and these receptors mediate the neuromodulatory actions of glutamate.

The metabotropic glutamate receptor family consists of at least eight members. These receptors have been traditionally divided into three main groups based on sequence similarity [9], as shown in Table 1. The genetic similarity of receptors within each group is reflected in the similar ligand-binding properties of members of each group relative to the other groups as well as similarities in signal transduction mechanisms [9]. The group I mGluRs include mGluRs 1 and 5. These receptors most commonly couple to activation

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**Fig. (1).** Expression of mGlu Subtypes in the Pain Neuraxis

Abbreviations: DRG – Dorsal root ganglia; RVM – Rostral Ventromedial Medulla; PAG – Periaqueductal grey; AM – Amygdala; HY - Hypothalamus.

of phospholipase C, but coupling to adenylyl cyclase and the ERK MAP Kinase cascades have also been reported [10-14]. The group II mGluRs include mGlu2 and 3, while group III mGluRs include mGlu4, 6, 7 and 8. Group II and group III mGluRs couple to inhibition of adenylyl cyclase in recombinant expression systems, and in the brain frequently function to modulate G protein-coupled inwardly rectifying potassium channels (GIRKS) or voltage-gated calcium channels (VGCC) [9, 15-17]. The genetic similarity of members of each group is also reflected in the similar pharmacological profiles of the different groups. Thus, there are group-selective agonists (such as 3,5-DHPG for group I, APDC for group II, and L-AP4 for group III) and similar group-selective antagonists have been identified (see Table 1). The designation of these functionally and pharmacologically similar groups provide a useful framework for addressing the functional roles of the different receptor subtypes in modulation of nociception. However, it should be noted that because many non-selective mGluR ligands have been used in the majority of the studies that will be discussed here, it is difficult to ascribe the activity to a specific receptor subtype.

The perception of pain can be profoundly modified by experience. In many ways, the plasticity in processing of nociceptive information is analogous to plasticity involved in learning and memory. For example, several models of chronic pain have been studied, and in almost every case,

plasticity at synapses in nociceptive processing centers requires activation of NMDA receptors [18, 19]. In many instances, this plasticity also requires or is modulated by mGluRs [20-22]. Multiple mGluR subtypes are expressed pre- and post-synaptically throughout the pain neuraxis, and recent studies have begun to elucidate the roles of mGluRs in regulating nociceptive transmission and plasticity.

Because NMDA receptors have been studied in great detail as potential mediators of nociceptive plasticity, they have been the most thoroughly studied as potential targets for the development of novel analgesics. NMDA receptor antagonists are being used in experimental therapies for the prevention and treatment of persistent pain following injury [23-29]. However, there are significant undesirable side effects associated with the use of non-subtype selective NMDA receptor antagonists due largely to the critical role of NMDA receptors in normal excitatory synaptic transmission throughout the nervous system. These side effects include fatigue, dizziness, psychosis, hyperactivity and in cases of higher levels of NMDA antagonists, amnesia and neuronal toxicity [24, 30]. It is interesting to note that activation of mGluRs enhances NMDA receptor function in dorsal horn neurons [31, 32], and this enhancement likely modulates NMDA receptor-dependent plasticity in the dorsal horn. Drugs designed to target specific mGluRs responsible for persistent alterations in nociception might have minimal effects on moment-to-moment excitatory transmission while

effectively modifying the abnormal elevation of transmission thought to underlie persistent pain states. Thus, mGluR antagonists might perform well clinically in intervention therapies at the time of injury or as analgesics during chronic pain states without the side effects inherent to NMDA receptor antagonists.

In this review, we will focus on the expression pattern of mGlu receptors in the pain neuraxis, as well as the neurochemical, electrophysiological and behavioral studies that have been performed over the last several years that suggest a potential role for mGluRs in the processing and modulation of nociceptive information. We will discuss the functions of these receptors in various types of acute and persistent pain models, and finish with an overall view of how these studies suggest strategies for the development of mGluR ligands for the treatment of pain associated with injury and disease.

### EXPRESSION PROFILE OF mGlu SUBTYPES IN THE PAIN NEURAXIS

An examination of the roles of mGluRs in nociceptive processing is aided by an understanding of the expression pattern of the different mGluR subtypes in regions of the nervous system involved in the processing or modulation of nociception (see Fig. [1]). In the peripheral sensory neurons themselves, several groups have identified the expression of all major groups of mGluRs. In group I, both mGlu1 and mGlu5 are expressed in dorsal root ganglia neurons (DRGs), and have also been localized to the peripheral terminals of these neurons [33-36]. Similarly, mGlu2/3-like immunoreactivity is seen in DRG somata and peripheral terminals [37, 38], but mGlu2/3 immunoreactivity is also prominent on presynaptic terminals of these neurons in the dorsal horn [39, 40], although it should be noted that

another study suggested the terminal staining for mGlu2/3 is not in primary afferents, but from local circuits in the cord [41]. mGlu4 and mGlu7 protein is seen on presynaptic terminals of sensory neurons in the dorsal horn [42-44]. mGlu6 is not expressed significantly outside the retina and olfactory bulb, and no studies have addressed the expression of mGlu8 in sensory neurons or spinal cord. However, some pharmacological experiments suggest that mGlu8 might be expressed on presynaptic terminals of sensory neurons in the cord [45]. No detailed studies of the expression of group III mGluRs on peripheral terminals have been reported.

Several studies have addressed the expression pattern of the mGluR subtypes in spinal cord. By Northern blot analysis, transcripts for mGlu1 and mGlu5 are expressed at high levels in the spinal cord [33, 46]. RT-PCR analysis suggests that this mRNA includes multiple splice variants of mGlu1 including 1a, 1b, 1d and 1f, and both the mGlu5a and 5b splice variants are expressed in the dorsal horn [14]. RNA for mGlu2, 3, 4 and 7 is present at much lower levels, and no RNA for mGlu6 or 8 was detected [46]. Immunohistochemical analysis has revealed that mGlu5 is strongly expressed in dorsal horn neurons [33, 40, 47], and mGlu5 immunoreactivity is associated with post-synaptic elements receiving inputs from presumed small myelinated afferents in the superficial layers of the dorsal horn [33, 40, 47]. There are conflicting reports on the expression of mGlu1a in the cord, with some reports suggesting expression of mGlu1a in the superficial dorsal horn [39, 48]. However, later studies utilizing different antibodies report mGlu1a staining only in deeper dorsal horn layers, and suggest that the original reports of mGlu1a in lamina I-II represented cross-reactivity of the antisera with mGlu5 [40, 49]. Immunoreactivity to antibodies raised against mGlu2/3 is also found in the dorsal horn [37, 40], and this staining is present in mGlu2-deficient mice [50], suggesting that mGluR3 is the main component of this staining. mGlu7

**Table 1. Metabotropic Glutamate Receptors can be Divided into Three Groups Based on Sequence Similarity. This Similarity is Reflected in Similar Coupling Mechanisms and Pharmacological Properties**

Group	Subtype	Signal Transduction	Agonists	Antagonists
I	mGlu1	PLC	DHPG	CPCCOEt, LY367385
	mGlu5	ERK		MPEP SIB-1757 SIB-1893
II	mGlu2	AC	APDC	LY341495
	mGlu3	GIRK VGCC	DCG-IV LY354740	
III	mGlu4	AC	L-AP4	MAP4 MSOP
	mGlu6	GIRK	L-SOP	
	mGlu7	VGCC	RS-PPG	
	mGlu8		DCPG	

immunoreactivity in the dorsal horn appears to be confined to terminals of nociceptive afferents [43]. mGlu4a antibodies strongly label the superficial dorsal horn, and this reflects not only the presynaptic terminals of sensory neurons mentioned above but also some postsynaptic labeling of dorsal horn neuronal dendrites [44].

The dorsal horn neurons project to a variety of brain regions involved in the processing of nociceptive information. Perhaps the most prominent projection is the spinothalamic tract (Fig. [1]), which originates primarily from neurons in laminae I and laminae V-VII and projects to the thalamus. In different thalamic nuclei, there is staining for mGlu1 [51-55], mGlu2/3 [37, 50], mGlu4 [56, 57], mGlu5 [58, 59] and mGlu8 [60]. The expression in the thalamus of mGlu5 and 8 is relatively weak, and mGlu2 levels are below detection [50]. The expression of mGlu1 and mGlu3 is particularly strong.

Another major projection of the dorsal horn nociceptive neurons is the midbrain reticular formation and periaqueductal grey (PAG), which is an important center for descending antinociceptive signals to the cord. The distribution of mGluRs in these midbrain regions has not been reported in detail. However, mGlu5 expression has been demonstrated in the PAG [61], and the involvement of mGlu5 in periaqueductal grey-mediated processing of antinociceptive information has been reported [62]. Staining for mGlu3 is also apparent in PAG but absent from the reticular formation [50]. Another processing center important for descending antinociception is the rostral ventromedial medulla (RVM), and little is known of the expression of function of mGluRs in this region. Together the PAG and RVM are responsible for the analgesic effects of opioids [63]. It will be important to conduct detailed localization studies for all of the mGluRs in these regions to provide information about the potential roles of these receptors in endogenous antinociception.

In addition, mGluRs are expressed in a number of other brain regions involved in the processing of nociceptive information, including the amygdala and association cortex. The role of mGluRs in these structures is an area of current unpublished efforts by a number of labs.

These studies demonstrate that multiple mGluRs are expressed in all of the major centers of the pain neuraxis. While this suggests that these receptors might be useful targets for modulating the processing of nociceptive information, it also highlights the complexity of the system that needs to be considered when evaluating the efficacy of drugs in a given assay. The consistent presynaptic localization of mGlu7 and postsynaptic localization of group I mGluRs might be informative in predicting the action of drugs targeted to these receptors. These predictions of course must be tested in detailed behavioral and electrophysiological studies, as outlined below, to determine the utility of mGluR ligands as modulators of nociception.

### Modulation of Acute Nociception by mGlu Receptors

A key question when one aims to develop analgesics is what is the function of the drug target in pain processing.

Ideally, one would want a drug that would leave acute pain sensation relatively intact, while dramatically reducing pathological pain hypersensitivity associated with, or following injury. Such a drug would prevent tissue damage that might occur in the absence of pain sensation, but would ameliorate the debilitating effects of chronic pain conditions. The mGluRs, given their role in modulation rather than mediation of synaptic transmission, might be ideal targets for the development of this type of drug. We will first consider whether these receptors mediate a component of acute nociceptive transmission.

Studies of the modulation of acute pain sensitivity by group I mGluRs have yielded differing results. For example, one study reported that 4C3HPG, a mixed group I mGluR antagonist / group II agonist can reduce the sensitivity to noxious tactile and thermal stimuli in rats [64]. Similarly, more selective mGlu1 antagonists reduce responses of primate spinothalamic tract (STT) neurons to noxious, but not innocuous mechanical stimuli [65], and antisense oligonucleotide-mediated reduction of mGlu1 expression in rat spinal cord leads to pronounced analgesia in a thermal test [66]. There is some debate over whether mGlu5 is involved in acute nociception in a manner similar to mGlu1. For example, the selective mGlu5 antagonist, MPEP, was found to significantly reduce the firing of ventroposterolateral (VPL) thalamic neurons (an area of prime importance in supra-spinal sensory processing) to noxious, but not innocuous, mechanical stimuli [67]. This effect likely involves modulation of spinal inputs, as MPEP had no effect on firing of VPL neurons when administered locally, but decreased ventral root potentials and wind-up in an *in vitro* spinal cord preparation. Consistent with this, systemically applied MPEP also reduces firing of thalamic neurons in response to noxious thermal stimulation [68]. Although these electrophysiological studies would suggest that mGlu5 plays a role in acute nociceptive processing, extensive behavioral studies have not supported this interpretation. Walker and coworkers showed clearly that very high systemic doses of MPEP have no effect on either thermal or mechanical pain thresholds in rats, while similar doses completely reverse certain types of hyperalgesia [69]. In addition, intrathecal or intraplantar administration of the mGlu5 antagonist SIB-1757 did not alter nociceptive behaviors in the thermal paw test [70]. Taken together, these results suggest that mGlu1, but not mGlu5, is involved in mediating acute nociceptive processing or transmission, presumably at the level of the spinal cord.

There are fewer studies examining the role of group II and group III mGluRs in acute pain. However, one study shows that agonists of group II mGluRs have no effect on nociceptive processing in the primate spinal cord, whereas a group III mGluR agonist produces a modest reduction in the firing of dorsal horn neurons in response to innocuous and noxious stimuli [71]. Detailed behavioral studies examining the effects of these drugs on basal nociception have not yet been reported, although one study showed that activation of group II mGluRs leads to increased noxious mechanical withdrawal thresholds in sheep [72]. In an abstract from Eli Lilly, reported at the 9<sup>th</sup> World Congress on Pain (August 22-27, 1999), selective mGluR2/3 agonists increased response latency time in the hot-plate test of acute pain, but

had no effect on tail-flick latency (Simmons *et al.*, abstract #101). More extensive studies utilizing the newer and more selective agonists and antagonists of group II and III mGluRs are therefore warranted to determine the role of these receptors in acute pain sensation.

## MODULATION OF PERSISTENT NOCICEPTION BY mGlu RECEPTORS

While mGluRs appear to have only a minor contribution to acute nociception, mGluRs may play a more significant role in the pathogenesis of chronic pain. For example, intrathecal administration of a group I mGluR agonist, DHPG, leads to spontaneous nocifensive behaviors, such as caudally oriented biting and licking, as well as thermal hyperalgesia and tactile allodynia [14, 72, 73]. The strongest case for mGluRs as targets for intervening in chronic pain conditions comes from a large number of studies using a variety of models of acute and chronic inflammation, detailed below.

### Inflammatory Pain –mGluRs in the Spinal Cord Dorsal Horn

#### Group I mGluRs

The first report of a potential role for metabotropic glutamate receptors in inflammatory pain conditions came in 1994 from the work of Neugebauer, Lucke and Schaible [21]. In this study, the authors found that following knee joint arthritis in rats, there is greatly enhanced excitability of dorsal horn neurons [21], and this effect seems to be largely mediated by mGluRs, as a non-selective mGluR antagonist, L-AP3, reversed established sensitization and prevent the development of arthritis-associated central sensitization [21]. These studies marked the beginning of the efforts to ascertain how mGluRs function in pain plasticity, but were hampered by the poor pharmacologic agents available at the time. Later studies, detailed below, have nonetheless supported these original results.

Group I mGluRs have been shown to be involved in a process known as central sensitization. Central sensitization can be loosely defined as an increase in responses of dorsal horn neurons following repeated or intense noxious stimuli [74]. The involvement of group I mGluRs has been shown in various inflammatory models including capsaicin-induced central sensitization in primates [65], arthritis-induced sensitization in rats [21], and stimulus-induced sensitization of dorsal horn neurons in turtles [75, 76] and rats [77]. In the primate studies by Neugebauer and Willis, the selective mGlu1 antagonist, CPCCOEt not only reduced the firing of STT cells to mechanical stimulation under basal conditions, but dramatically reduced the hyper-responsiveness of these STT cells during capsaicin-evoked sensitization, indicating a complete reversal of sensitization. These results clearly implicate group I mGluRs, and particularly mGlu1, in the development of central sensitization associated with inflammation. The mechanisms through which activation of group I mGluRs in the spinal cord leads to central sensitization likely involve various forms of synaptic

plasticity, including LTP and LTD [78, 79]. Similar to these studies in the cord, group I mGluR activation can either induce or potentiate the induction of LTP in different brain regions [80-86], and under other conditions can induce LTD [87, 88].

The formalin model is frequently used in the study of inflammatory pain states in rodents [89]. Injection of formalin in the hind paw results in a typical biphasic nociceptive response [90]. The first phase, usually lasting less than 5 min, occurs a few seconds after formalin injection and is characterized by intense licking or lifting of the injected paw. This phase is due to acute stimulation of nociceptors. The second phase is characterized by licking and lifting of the injected paw, beginning about 20 min after formalin injection and lasting until approximately 50 min after injection. This second phase is thought to involve central sensitization of dorsal horn neurons as well as peripheral sensitization associated with the inflammation [91]. A number of studies have found that group I mGluRs contribute to formalin-induced hyperalgesia. Intrathecal (i.t.) administration of group I mGluR agonists have been shown to enhance the formalin test second phase nociceptive responses [92], whereas antagonists of group I mGluRs inhibit the second phase [14, 92]. This effect is seen with antagonists selective for either mGlu1 (CPCCOEt) or mGlu5 (MPEP), suggesting that both receptor subtypes may be involved in mediating the formalin second phase [14]. Similarly, group I mGluR antagonists can reverse carrageenan-induced hypersensitivity to mechanical and thermal stimuli [64].

Several group I-selective antagonists reduce secondary hyperalgesia in rats [93]. The acute knee joint inflammation model demonstrates both primary hyperalgesia within the inflamed area and secondary hyperalgesia in tissue surrounding the inflamed area. Spinal administration of the mGluR1/5 antagonist, LY393053, and the mGluR1-selective antagonist LY367385 reduced secondary thermal hyperalgesia, consistent with the view that secondary hyperalgesia is thought to result from the sensitization of dorsal horn neurons [93]. None of the antagonists affected baseline paw withdrawal latencies (acute pain) or knee-joint inflammation. Taken together, these results suggest that spinal group I mGluRs may play an important role in nociceptive hypersensitivity associated with inflammation. Consistent with these studies indicating a role for group I mGluRs in sensitization, another study has found that a general group I mGluR antagonist, LY393053, reduces behavioral responses in a visceral pain model [94].

#### Group II mGluRs

The role of group II mGluRs in regulating nociceptive transmission in the spinal cord is less clear. Given the expression of these receptors in the dorsal horn and/or on primary afferent terminals [37, 95] and their role in modulating excitatory [96] and inhibitory [97] transmission in the cord, it seems that these receptors could be exploited to modify sensory transmission. (S)-4C3HPG, an agonist at mGlu2 and 3 and an antagonist at group I mGluRs, has been shown to reduce the second phase of the formalin test [92]. However, the more selective group II agonist, DCG-IV has

no effect in this model in one study [92], while three different mGlu2/3 agonists developed by Lilly (LY354740, LY379268 and LY389795) attenuated formalin-induced paw licking behavior following subcutaneous administration (Simmons *et al.*, abstract #101, 9<sup>th</sup> World Congress on Pain, August 22-27, 1999). Furthermore, these highly selective mGlu2/3 agonists from Lilly also reversed carrageenan-induced thermal hyperalgesia, but not mechanical allodynia (Jones *et al.*, abstract #100, 9<sup>th</sup> World Congress on Pain, August 22-27, 1999).

There is some evidence that under inflammatory conditions, group II mGluRs may be upregulated. For example, Stanfa and Dickenson found that 1S,3S-ACPD, an agonist at group I and group II mGluRs, can inhibit discharges of dorsal horn neurons in response to C fiber stimulation following carrageenan-induced inflammation, but not in control animals [98]. This could reflect increased expression of group II mGluRs or increased coupling of these receptors in the inflamed state. Furthermore, the group II mGluRs involved here could be localized on dorsal horn neurons or on central terminals of primary afferents. Interestingly, Neugebauer and colleagues reported that activation of group II mGluRs in the cord has no effect on the firing of dorsal horn neurons in response to graded mechanical stimuli under normal conditions, but following capsaicin-induced sensitization, group II agonists significantly depress firing of STT cells in response to light and moderate stimulation [71]. This study suggests that agonists of group II mGluRs might be effective at reducing hyperalgesia and allodynia while leaving normal nociception intact, and again points to a functional upregulation of spinal cord group II mGluRs associated with central sensitization.

Consistent with the idea that inflammation leads to increased expression of group II mGluRs in the cord, Sarah Boxall and coworkers reported that UV irradiation-induced peripheral inflammation causes an increase in the expression of mGlu3 mRNA in the dorsal horn [95]. These studies suggest that mGluRs are strongly regulated by conditions in which nociception is enhanced. Interestingly, Ferdinando Nicoletti and coworkers have recently shown that L-acetylcarnitine, an analgesic used to treat neuropathic pain [99] exerts its actions by causing an upregulation of mGlu2 in the spinal cord of rats [100]. These findings, particularly with highly selective group II agonists, suggest that mGlu2/3 agonists may be efficacious in inflammatory pain conditions, and further suggests a novel aspect of mGluR regulation during inflamed states that makes these receptors very interesting candidates for analgesic development.

### **Group III mGluRs**

The group III mGluRs, mGlu4, mGlu7 and mGlu8 all appear to modulate nociceptive input to the cord. mGlu4 and 7 are expressed in the dorsal horn [43, 44, 101, 102], and the group III mGluR agonist, L-AP4, reduces primary afferent transmission [96], as does a relatively selective mGlu8 agonist, (S)-3,4-DCPG, indicating that mGlu8 specifically may regulate transmission at this synapse [45]. The final group III mGluR, mGlu6, is not expressed in these areas [103].

The specific role of group III mGluRs in regulating nociceptive hypersensitivity was most clearly illustrated by Neugebauer and Willis [71]. Similar to what they reported for group II mGluRs, this group reported that capsaicin-induced hypersensitivity to non-noxious stimuli is reversed by the group III mGluR agonist, L-AP4. Unlike group II mGluR agonists, which had no effect in the basal state, group III mGluR agonists also reduced responses to mild, moderate and intense mechanical stimulation under basal conditions. Thus, while these receptors are able to reverse tactile allodynia, they also reduce basal nociceptive transmission.

### **Inflammatory Pain-mGluRs Expressed on Primary Afferents**

Several mGluRs are expressed in primary afferent neurons [34-36, 38]. Until recently, it was believed that these receptors likely functioned to modulate transmitter release from the central terminals of primary afferents, and this may be the case in part. However, several recent studies indicate that mGluRs expressed on peripheral terminals of sensory neurons can also modulate nociception. For example, activation of peripheral group I mGluRs by intraplantar injection of DHPG increases sensitivity to mechanical [35, 36] and thermal [34] stimuli in naïve animals. This hyperalgesia is of interest in light of recent evidence suggesting that glutamate is released in response to inflammation [104, 105]. To test the role of these peripheral mGluRs in inflammatory hyperalgesia, antagonists of group I mGluRs were injected into the plantar skin in rodents, and the effects of these antagonists were evaluated in normal and inflamed animals. Intraplantar injection of the mGlu5 antagonist, MPEP, was found to be analgesic in both the complete Freund's adjuvant [36] and formalin [34] models of inflammatory hyperalgesia. The functional effects of peripheral group II mGluRs have not yet been reported. The possible analgesic effects of blocking or activating peripheral mGluRs selectively could provide a safer route for treatment of pain conditions by avoiding undesirable CNS side effects [106].

### **Inflammatory Pain - Supraspinal mGluRs**

In addition to the clear roles played by mGluRs in primary afferents and dorsal horn neurons, there are a number of studies suggesting that mGluRs expressed in supraspinal centers are also critically involved in regulating nociception and nociceptive plasticity. In particular, several studies have focused on mGluRs in the thalamus and PAG.

There are a large number of papers by Salt and colleagues that demonstrate functional effects of multiple mGluR subtypes on thalamic processing of somatosensory (including nociceptive) information [107-112]. Some of these reports do not focus specifically on nociception and thus the extent to which these findings can be generalized and applied to other types of sensory stimuli, including nociceptive signals, is not clear. However in total these studies have demonstrated that somatosensory information can be modulated by all pharmacological groups of mGluRs.

Bordi and Ugolini have examined the role of mGlu5 in nociceptive responses of thalamic neurons in the VPL. This study shows that the firing of VPL neurons in response to noxious mechanical stimulation is reduced by systemically applied MPEP, but that this effect of MPEP is not mediated locally in the thalamus [67]. This is in contrast to the effect of MPEP on thalamic processing of noxious thermal stimuli, where local MPEP application reduces firing of VPL neurons to immersion of the receptive field in 52°C water [110].

Group II mGluRs also appear to modulate thalamic processing of nociceptive information. Intrathalamic (VPL) injection of the group II mGluR antagonist, EGLU, causes a transient reduction in nociceptive behaviors following CFA-induced monoarthritis [113]. It is likely that group II mGluRs in the thalamus exert an inhibitory effect on release of GABA. In this study, the authors did not address whether EGLU had similar effects in naïve or vehicle treated animals. Therefore, it is not clear whether this reflects a tonic stimulatory effect of group II mGluRs on nociceptive perception or a particularly upregulated effect following inflammation, as was discussed above.

The periaqueductal grey region is an extremely important processing center for nociceptive information, and mediates descending inhibition of nociception [114]. In the PAG, several studies have addressed the effects of mGluR antagonists and their role in inflammatory pain modulation. Evidence indicates that multiple mGluRs localized in the PAG contribute to tonic modulation of nociception, and mediate the antinociceptive properties of cannabinoids in the PAG [62]. The PAG is also known to modulate formalin-induced nociceptive behaviors [115], and activation of group I mGluRs in the PAG causes a dose-dependent blockade of the second phase of the formalin test [116]. Thus, group I mGluRs in the PAG have exactly the opposite effect of group I mGluRs in the spinal cord dorsal horn with respect to modulation of the formalin test. This type of competition between different regions of the CNS is a critical consideration when evaluating the potential efficacy of analgesics. It will be important to determine whether the hyperalgesic effects of MPEP in the PAG is more important than the analgesic effects mediated by actions on the spinal cord and nociceptors in the net effect of this and related compounds on inflammatory hyperalgesia.

The studies discussed above provide a fairly consistent view of the specific roles played by the different pharmacological classes of mGluRs in the regulation of nociception. In general, activation of group I mGluRs enhances sensitivity to noxious and innocuous stimuli, suggesting a role for these receptors in the establishment of hyperalgesia and allodynia. This is supported by the finding that antagonists of group I mGluRs can prevent the induction of chronic pain conditions, and importantly these drugs can reverse established hyperalgesia. The exception noted above with regards to the PAG effects is an important caveat. The group II and group III mGluRs appear to be involved in anti-nociceptive effects of glutamate. The interesting finding that these receptors are functionally upregulated following inflammation may be valuable in designing treatments for inflammatory hyperalgesia.

## MODULATION OF NEUROPATHIC PAIN BY mGlu RECEPTORS

Peripheral nerve injury in rodents has been used as an experimental model to mimic symptoms associated with neuropathic pain, notably tactile allodynia and thermal hyperalgesia [117, 118]. Following injury, the peripheral nerves exhibit spontaneous, persistent afferent discharges, leading to a sensitization in the spinal cord that drives the development of neuropathic pain. The mechanisms underlying the sensitization within the spinal cord are complex, and likely involve changes in cell-surface receptor and ion channel expression, activation of intracellular signaling cascades and glial proliferation [132]. This central sensitization leads to heightened responsiveness to noxious and innocuous input. It is important to note that neuropathic pain can also develop following central neuron lesions, including spinal cord injury [119], with patients exhibiting similar profiles in the clinic to peripheral nerve injury.

As we have discussed above, glutamate is the predominant neurotransmitter released from primary afferents, and likely plays an important role in central sensitization via activation of ionotropic and metabotropic glutamate receptors on second order neurons to enhance nociceptive transmission to higher centers. Consistent with this, glutamate levels are elevated in the superficial dorsal horn of rats after chronic ligation of the sciatic nerve [120]. Intrathecal injection of the group I agonist, DHPG, recapitulates some aspects of central sensitization, namely thermal hyperalgesia and tactile allodynia [73] that are observed in neuropathic pain. It is not surprising therefore that several studies support the findings that blockade or down regulation of group I mGlu receptors reduces aspects of central sensitization observed following nerve injury. The weak mGlu1/5 antagonist 4-carboxyphenylglycine (4CPG) failed to reverse either tactile allodynia or cold hyperalgesia by intrathecal administration in rats following sciatic nerve constriction injury [121]. However, in the same study, 4CPG attenuated the development of mechanical allodynia and cold hyperalgesia when administered *prior* to insult. Researchers at Eli Lilly recently presented data that support a role for mGlu1. The mGlu1 subtype-selective antagonist, LY367385, reversed pain behaviors in several persistent pain models, including mechanical allodynia in the spinal nerve ligation (Chung) model in rats when administered via an intracisternal route (Simmons *et al.*, Poster 53.15 and Kalra *et al.*, Poster 53.16, Society for Neuroscience meeting, 2001) supporting a role of central mGlu1 in neuropathic pain models.

Spinal administration of antisense oligodeoxynucleotides targeted to mGlu1 to attenuate receptor expression supports a role of mGlu1 in various pain models [66, 122]. Antisense oligonucleotide-mediated knockdown of mGlu1 in the spinal cord (and possibly the DRG and brain) of neuropathic rats reversed cold hyperalgesia to pre-injury levels, and partially reversed thermal hyperalgesia and mechanical allodynia [122]. Abrogation of nerve-injury-induced hyperalgesia and allodynia with mGlu1 antisense was observed with treatment pre- and post-nerve injury [122], suggesting a role of mGlu1 in the initiation and maintenance of neuropathy. Furthermore, the spinal injection of antibodies targeting the

intracellular epitopes of mGlu1 and mGlu5 reduced aspects of neuropathic pain in rats following chronic constriction injury of the sciatic nerve, without affecting acute nociception [123]. The mechanisms by which these antibodies achieved efficacy are unclear (the epitopes for the antibodies are intracellular), although receptor specificity was observed.

The contribution of spinal mGlu5 in the manifestation of tactile allodynia and thermal hyperalgesia after spinal nerve ligation was evaluated using SIB-1757, an mGlu5-selective antagonist [124]. In spinal nerve-ligated rats, intrathecal administration of SIB-1757 partially reversed tactile allodynia, measured by paw withdrawal thresholds to probing with von Frey filaments [70]. However, in the thermal hyperalgesia test, SIB-1757 produced a full reversal, measured by paw withdrawal latencies to radiant heat applied to the plantar aspect of the hindpaw following administration either spinally or locally to the injured paw [70]. In the Seltzer model involving partial ligation of the sciatic nerve [125], mechanical hyperalgesia was not altered by systemic administration of MPEP at doses that were effective in inflammation-induced mechanical hyperalgesia [69].

There are a number of studies that suggest an important role of mGluRs in pain associated with spinal cord injury. Following spinal cord injury (SCI), there is an increase in extracellular excitatory amino acid (EAA) concentrations, resulting in glutamate receptor-mediated excitotoxicity and central sensitization [126]. Intraspinally administered AIDA (a weak group I antagonist) decreased extracellular glutamate within the spinal cord when administered to rats immediately after spinal cord contusion injury and attenuated the development of mechanical but not thermal allodynia [126]. Furthermore, changes in expression levels of mGluRs occur both spatially and temporally after SCI in rats and parallel the development of mechanical allodynia and thermal hyperalgesia [49]. These changes were long lasting in some cases: mGluR1 was increased over control levels in segments rostral and caudal by postsurgical day 7 and remained elevated for at least 60 days, and group II mGluRs (immunoreactive mGluR2/3) were decreased compared to control levels from day 7 through day 60. Understanding the role of these expression changes after SCI may give insight into mechanisms underlying the development of chronic central pain.

In summary, these data suggest an involvement of mGlu1, and to a lesser extent, mGlu5 in the mediation of aspects of neuropathic pain. Blockade of mGlu1 with selective antagonists might be expected to attenuate cold hyperalgesia, heat hyperalgesia and mechanical allodynia in nerve injury models. Data for this prediction is currently lacking due to the absence of potent, selective, systemically active mGlu1 antagonists. The role of mGlu5 in neuropathic models has been empirically determined with selective antagonists. The conclusions of these studies suggest a role for mGlu5 in thermal hyperalgesia, consistent with reports that this receptor may be associated with afferent C-fibers [14, 36, 127], and a less impressive effect on tactile allodynia, likely to be mediated by large A fiber input.

The mechanisms by which stimulation of group I mGluRs might play a role in neuropathic pain are unclear. It is known however, that stimulation of group I mGlu receptors activates protein kinase C (PKC) [128] and MAP kinase [12-14] signal transduction pathways. A large body of data supports a role of PKC in nociception. Coincident with nociception, both noxious chemical stimulation and nerve injury produces an increase in PKC translocation in the spinal cord of rats [129, 130, 131]. Activation of PKC by spinal administration of phorbol esters enhances formalin-induced nociception in rats, while selective PKC inhibitors reduce nociceptive responses to formalin [132, 133]. Furthermore, PKC -knockout mice showed a significant reduction in mechanical and thermal allodynia following nerve constriction injury and the formalin test [134]. Stimulation of group I mGlu receptors activates PKC, and group I antagonists or mGlu1 antisense reduce [<sup>3</sup>H]-PDBu binding in lumbar spinal cord after nerve injury coincident with an attenuation of mechanical and cold allodynia [122, 135].

A change in expression levels of proteins that play a role in nociception might be expected to occur in neuropathic pain models. Few studies have compared mGlu receptor mRNA or protein expression changes following neuropathy. With Western blots for mGlu1 and mGlu5, receptor levels did not show an appreciable change in lumbar spinal cord or thalamus/periaqueductal grey following nerve constriction compared to sham surgery controls [122]. In a tibial nerve injury model, mRNA for mGlu1 in the DRGs was down-regulated in both sham operated and nerve-injured animals [136]. The authors suggest that down-regulation of these receptors by peripheral tissue damage could induce a loss of autoreceptor function, resulting in the hyperglutamatergic condition associated with chronic pain.

There is, surprisingly, no published data with non-selective or selective group II or group III mGlu receptor ligands in peripheral neuropathic pain models. In a chronic central model, the rat SCI, the group II-selective agonist, APDC, and the group III agonist, L-AP4 attenuated the development of mechanical, but not thermal hyperalgesia, while having no measurable neuroprotective effect [137].

## **EFFECTS OF mGlu LIGANDS ON MORPHINE TOLERANCE AND DEPENDENCE**

Morphine is commonly used for the management of pain, although in a limited manner due to potential tolerance and dependence with chronic use, as well as side effects associated with morphine (constipation, nausea, sedation). Furthermore, in the clinic, neuropathic pain is often only partially relieved by high doses of opioids [1, 4-6]. NMDA receptor antagonists [138] and mGlu receptor antagonists [139] attenuate the development of tolerance and dependence if co-administered with morphine. Thus, group I mGlu receptor antagonists and group II mGlu receptor agonists [140] decrease opioid dependence, measured by precipitated withdrawal symptoms. Furthermore, in a neuropathic pain model, the ability of intrathecal morphine to increase tail-flick latencies was reduced relative to sham-operated animals [122]. However, antisense to mGlu1 restored the ability of

morphine to produce a normal analgesic response. Thus, knockdown of mGlu1 at the spinal level prevented the development of morphine insensitivity in neuropathic rats.

The mGlu2/3-selective agonist, LY354740 inhibited the development of tolerance to antinociceptive effects of morphine, but not fentanyl, in the mouse tail-flick test. LY354740 did not affect the tail-flick antinociceptive response alone, or the acute antinociceptive effect of morphine [141]. Furthermore, pretreatment with LY354740 suppressed the severity and occurrence of many naltrexone-precipitated morphine-withdrawal signs [142]. One potential mechanism through which LY354740 is able to decrease opiate withdrawal behaviors might be through a reduced firing rate of locus coeruleus (LC) neurons [142]. Thus, mGlu1 antagonists and mGlu2/3 agonists could be useful as an adjunct to opiates to prevent (or reverse) tolerance.

**ROLE OF GLIA IN PERSISTENT PAIN**

Dogma suggested that within the CNS, glia played no role in cell-to-cell signaling because they lack axons, but rather acted as static constituents serving primarily support functions. It is now clear that spinal cord glia (microglia and astrocytes) can amplify pain states following activation, releasing pro-inflammatory cytokines (reviewed by Watkins et al [143]). Microglia and astrocytes can be activated under many conditions including spinal infection, cutaneous inflammation and following peripheral nerve injury. Likely chemical activators are substance P, glutamate and ATP, released from sensory nerve terminals in the dorsal horn. Activated glia release substances that excite spinal neurons, such as reactive oxygen species including NO, arachidonic acid metabolites, glutamate, growth factors, and the pro-inflammatory cytokines interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF).

Astrocytes express mGlu receptors, although the expression pattern appears to depend on the species, the

CNS region, and also the culture conditions. For example, Condorelli and coworkers detected mRNA for mGlu3 and mGlu5 only in primary type-1 astroglial cultures derived from cerebral cortex and striatum [144]; similarly, Aronica and coworkers identified mGlu2/3 and mGluR5 in reactive astrocytes and microglia obtained from rat hippocampus [145]. Conversely, mGlu5 was poorly expressed in pure rat spinal cord astrocyte cultures, but mGlu1 and mGlu2/3 immunoreactivity was detected [146]. Thus cultured astrocytes from different parts of the CNS exhibit different patterns of mGluR expression. These findings of multiple mGluR subtypes expressed in glia in the cord allow for the possibility that activation of these receptors could be a critical regulator of glial involvement in hyperalgesia. Further studies in this area are warranted.

**CONCLUSIONS**

**Are mGlu Receptors Viable Targets for Development of Antinociceptive Agents?**

In the clinic, one would wish to have a drug that would not affect normal nociception, which serves an important protective role in limiting tissue injury, but would specifically reduce the enhanced pain sensitivity observed following injury or disease. Furthermore, if drugs are to be effective as analgesics, they should be effective at reversing existing conditions, as it is rare that drug treatments can be utilized close enough to the time of injury to prevent sensitization. Therefore, the finding that agonists of mGluRs seem to have enhanced efficacy following the induction of chronic pain conditions is an important finding.

The aim in drug discovery is to develop compounds that have confirmed efficacy and a favorable therapeutic index such that the compounds are without appreciable side effects at efficacious doses. The selection of validated drug targets is extremely difficult; in many cases for example, there is insufficient efficacy or little separation between efficacy and

**Table 2. Summary of the Activity of mGlu Ligands in Animal Models of Pain**

Pain Model	Pain Modality	Group I Antagonists		Group II Agonists	Group III Agonists
		mGlu1	mGlu5	mGlu2, mGlu3	mGlu4, mGlu7, mGlu8
Acute	Thermal Noxious			× /	
	Mechanical Noxious			×	
Persistent	Carrageenan				
	CFA				
	Formalin				
	Capsaicin				
	Kaolin/Carrageenan				
Neuropathic	Touch-evoked Allodynia		×		
	Thermal Hyperalgesia				

- Data supports an antinociceptive effect; × - Data do not support an antinociceptive effect; blank fields indicate there is no data supporting an effect, or lack thereof.

adverse side effects. These targets are therefore not likely to be commercially successful.

Validation of mGlu receptors as *bona fide* drug targets has not yet been obtained due to the paucity of potent, selective mGlu receptor subtype ligands with good brain penetration. Thus far, only agonists and antagonists for mGlu2/3 and antagonists for mGlu5 have been characterized in detail. The data to date, summarized in Table 2, suggests that activation of mGlu2/3 may be efficacious in inflammatory pain, and as an adjunct to reduce tolerance of morphine. Evaluation of selective mGluR2/3 agonists in rodent models of neuropathic pain is certainly warranted. Likewise, mGlu5 antagonists are active in inflammatory pain models, but do not show broad-spectrum activity in neuropathic pain models. Agents acting at mGlu2/3 and mGlu5 may therefore prove useful in the clinic to treat a subset of chronic pain conditions associated with inflammation.

In contrast, the literature supports a role of mGlu1 in inflammatory pain and neuropathic pain states, and it is perhaps the strongest mGlu candidate to date as a pain target. The lack of systemically bioavailable antagonists has prevented the examination of the potential side effects of antagonizing mGlu1. The mGlu1 knockout mice show profound effects on motor coordination [147]; a critical component of developing mGlu1 antagonists will be to evaluate locomotor and coordination side effects in parallel to pain models. Furthermore it would be interesting to test potent, but non-selective mGlu1/5 antagonists in models of pain and for side-effect liabilities.

In summary, the above discussion of the wide and varied distribution of different mGluR subtypes along with the sometimes conflicting findings regarding the role of different mGluRs using different preparations highlights the need for detailed behavioral analysis of the effects of highly potent and selective mGluR ligands. These types of studies allow one to determine the net effect of blocking a given receptor subtype throughout the organism, whereas local administration studies may suggest promising targets that prove unusable due to multiple counteracting sites of action in the brain. It is also possible, however, given our discussion above regarding the peripheral mGluR expression in sensory neurons, that an exclusive emphasis on systemically active drugs could miss a therapeutically effective drug because of unwanted central effects. The development of potent, orally active compounds that target mGluRs has not proven as difficult as those that have plagued the opiate receptor field for years. The studies discussed above point to the exciting possibility that new compounds acting at mGluRs could be used in the clinic to treat a wide variety of pain conditions associated with injury and disease.

#### ACKNOWLEDGEMENTS

We would like to thank Drs. Aldric Hama, Mark Urban and Bill Martin for providing input to the manuscript. Work in the lab of R.G. is funded by grants from NIMH (MH60230) and NARSAD.

#### ABBREVIATIONS

AMPA	=	-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
CFA	=	Complete Freund's adjuvant
DRG	=	dorsal root ganglia
ERK	=	extracellular signal-regulated kinase
GIRK	=	G-protein activated inwardly rectifying K <sup>+</sup> channel
iGluRs	=	ionotropic glutamate receptors
IL	=	interleukin
i.t.	=	intrathecal
LC	=	locus coeruleus
LTD	=	long term depression
LTP	=	long term potentiation
MAPK	=	mitogen-activated protein kinase
mGlu	=	metabotropic glutamate receptor
NMDA	=	N-methyl-D-aspartate
PAG	=	periaqueductal grey
PDBu	=	4-phorbol-12,13-dibutyrate
PKC	=	protein kinase C
RVM	=	rostral ventromedial medulla
SCI	=	spinal cord injury
SS	=	somatosensory cortex
STT	=	spinothalamic tract
TNF	=	tumor necrosis factor
UV	=	ultraviolet
VGCC	=	voltage-gated calcium channels
VPL	=	ventroposterolateral

#### CHEMICAL NAMES

4C3HPG	=	4-carboxy-3-hydroxyphenylglycine
4CPG	=	4-carboxyphenylglycine
ACPD	=	1-aminocyclopentane-1,3-dicarboxylic acid
AIDA	=	1-aminoindan-1,5-dicarboxylic acid
AP4	=	2-amino-4-phosphonobutyric acid

- APDC = 4-aminopyrrolydine-2,4-dicarboxylic acid
- CHPG = 2-chloro-5-hydroxyphenylglycine
- CPCCOEt = 7-(hydroxyimino)cyclopropan[b]chromen-1-carboxylic ethyl ester
- DCG-IV = (2S,1'R,2'R)-2-(2',3'-dicarboxycyclopropyl)glycine
- DCPG = 3,4-dicarboxyphenylglycine
- DHPG = 3,5-dihydroxyphenylglycine
- EGLU = (2S)- $\alpha$ -ethylglutamate
- HPG = 3-hydroxyphenylglycine
- L-AP3 = L-amino-3-phosphono-propionate
- LY341495 =  $\alpha$ -9'-xanthenylmethyl-2-(carboxycyclopropyl)glycine
- LY354740 = (+)-(1S,2S,5R,6S)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid
- LY367385 = 2-methyl-4-carboxy-5-hydroxyphenylglycine
- LY379268 = (-)-2-oxa-4-aminobicyclo[3.1.0]hexane-4,6-dicarboxylic acid
- LY389795 = (-)-2-thia-4-aminobicyclo[3.1.0]hexane-4,6-dicarboxylic acid
- LY393053 = 2-amino-2-(3-carboxycyclobutyl-3-(9-thioxanthyl)propionic acid)
- MAP4 =  $\alpha$ -methyl-L-AP4
- MPEP = 2-methyl-6-(phenylethynyl)pyridine
- MSOP =  $\alpha$ -methylserine-O-phosphate
- PPG = 4-phosphonophenylglycine
- SIB-1757 = 6-methyl-2-(phenylazo)-3-pyridinol
- SIB-1893 = 2-methyl-6-(phenylazo)pyridine
- SOP = serine-O-phosphate
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