Glycine receptors and glycine transporters: targets for novel analgesics?

Hanns Ulrich Zeilhofer1,2 · Mario A. Acuña1 · Jacinthe Gingras3 · Gonzalo E. Yévenes4

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Chronic pain is an unmet clinical need

World-wide, chronic pain affects about one in five individuals [1]. In particular chronic neuropathic pain is a difficult-to-treat medical condition. The most widely used analgesics, the cyclooxygenase inhibitors, which include aspirin, ibuprofen or diclofenac, and paracetamol or metamizole, are poorly effective against neuropathic pain. Opioids, the second widely used class of analgesics, are effective, but their use in patients with chronic non-malignant pain is highly problematic [2] and has caused major health problems in the US. Chronic inflammatory pain responds typically well to cyclooxygenase inhibitors [non-selective non-steroidal anti-inflammatory drugs (NSAIDs) and cyclooxygenase-2-specific coxibs], but severe and potentially life-threatening adverse events such as gastrointestinal bleeds and myocardial infarction severely limit their long-term use [3]. Drugs that are frequently used in chronic neuropathic pain conditions include gabapentin and pregabalin, which target voltage-gated calcium channels, in addition to compounds such as duloxetine that inhibit the re-uptake of noradrenaline and serotonin and thereby enhance endogenous pain control. Although clinical trials have clearly documented the therapeutic efficacy of these drugs, typically only about one out of seven patients shows clear benefits [4]. Chronic pain, thus, remains a major therapeutic challenge prompting for renewed drug discovery efforts.

Symptoms of chronic pain include hyperalgesia, allodynia and spontaneous pain [5]. Hyperalgesia defines an increased sensitivity to noxious (painful) sensory stimuli, such as
heat, cold, or intense mechanical stress. It results from a
sensitization either of peripheral nociceptors (i.e., of nerve
fibers and cells that are only activated by painful stimuli)
and/or of central neuronal pathways, which can occur via
enhanced transmission from nociceptors to second-order
neurons located in the spinal dorsal horn [6]. A second fre-
quent symptom of chronic pain is allodynia, which describes
painful sensation evoked by stimulation of non-nociceptive
nerve fibers, in particular of touch-sensitive fibers. Allodynia
is, therefore, always of central origin. A third characteristic
of chronic pain is spontaneous pain, occurring in the absence
of any sensory input. Its physiological correlate is likely the
spontaneous discharge of peripheral and/or central neurons
in the pain pathway(s). A plethora of molecular mechanisms
have been identified that can contribute to these symptoms
[7], but their specific contribution to defined human patient
pain states still remains obscure. It is, however, remarkable
that all three key symptoms occur when synaptic inhibition
in the spinal dorsal horn is compromised [8]. Not surpris-
ingly, one of the newly emerging targets against chronic pain
is the spinal glycinergic system, which together with the
GABAergic system exerts a powerful inhibitory control over
spinal nociception. Figure 1 illustrates key elements of the
spinal glycinergic system.

**Fig. 1** The glycinergic system. a Inhibitory (strychnine-sensitive)
glycine receptors are pentameric chloride-permeable ion channels. b
Each subunit has four transmembrane segments and two cys-loops
in the extracellular domain. c Five genes encode for glycine receptor
subunits. The α4 subunit gene (GLRA4) is a pseudogene in humans.
d Sagittal section through the mouse brain stained with an antibody
against GlyT2 illustrates the distribution of glycinergic axon termi-
nals (modified from ref. [10]). e Distribution of the glycine receptor
α3 subunit (green) in the mouse spinal cord (modified from ref. [14]).
Red, distribution of gephyrin. d and v indicate dorsal and ventral
direction, respectively. f Contribution of glycine receptors to the total
evoked IPSC in excitatory neurons of the superficial mouse spinal
dorsal horn. In the majority of neurons, the contribution of glycine
receptors to the IPSC amplitude dominates over that of GABA. Modifi-
ced from ref. [8]
Glycine and GABA in central pain control

GABA and glycine are the two fast inhibitory neurotransmitters in the adult spinal cord and brainstem. Neurons that synaptically release glycine or GABA (i.e., glycinergic or GABAergic neurons) are found both in the ventral and the dorsal part of the spinal cord. Glycinergic neurons of the dorsal horn are concentrated in the deep dorsal horn (lamina III and deeper), which is mainly innervated by myelinated low-threshold (tactile) sensory nerve fibers [9, 10]. Some glycineric neurons are located in lamina I, which receives sensory input mainly from non-myelinated high threshold (nociceptive) sensory nerve fibers. Lamina II contains only few glycineric neurons. Most spinal glycineric neurons co-release glycine together with GABA from the same presynaptic vesicles [11–13]. Despite the relative absence of glycineric somata in lamina II, neurons located in this lamina still express glycine receptors at high density [14] and also receive prominent glycineric input, likely through innervation from neighboring laminae and axons descending from the rostral ventromedial medulla [8, 15–17].

Early work from the 1980s indicated a major role of inhibitory glycine receptors in the spinal control of nociception [18–20]. These studies used intrathecal injections of the competitive glycine receptor antagonist strychnine in rats to show that blockade of spinal glycine receptors induces a profound agitation of the injected animals and a sensitization to tactile stimuli. Although subconvulsive doses of strychnine were used, such studies did not permit to restrict the actions of strychnine to the sensory (dorsal) spinal cord and spare ventral horn glycine receptors. More recent work has employed virus-based methods to locally and specifically ablate, silence, or activate glycineric neurons of the mouse spinal dorsal horn [8]. These studies revealed that impaired inhibition by glycineric dorsal horn neurons rendered mice hypersensitive to a broad range of noxious sensory stimuli including heat, cold and mechanical stimuli and induced signs of spontaneous discomfort. Conversely, sensitivity to these stimuli was strongly reduced when dorsal horn glycineric neurons were activated using a chemogenetic approach. Other evidence indicates that glycineric neurons become activated under acute and even more pronounced under chronic pain conditions [21]. Furthermore, one of the typical and most bothersome symptoms of neuropathic pain is allodynia, or touch-evoked pain [5]. Allodynia is likely due to the activation of nociceptive projection neurons by polysynaptic input from touch-sensitive (non-nociceptive) sensory fibers. Increasing evidence suggests that inhibitory neurons in the deep dorsal horn normally prevent this activation and that their activity is compromised in neuropathic and, possibly equally, in inflammatory pain states [22]. Restoring this inhibition would hence target a major symptom of chronic pain.

Several groups have demonstrated that synaptic inhibition becomes diminished in rodent models of chronic inflammatory or neuropathic pain [23–25] (Fig. 2). This reduction in inhibition occurs apparently through multiple mechanisms, of which some affect both GABAergic and glycineric inhibition, whereas others are specific for the glycineric component (for a review on these mechanisms see [26]). In the superficial dorsal horn, responsiveness of glycine receptors to their physiological agonist glycine is reduced by the inflammatory cytokine prostaglandin E2 (PGE2). PGE2 induces a specific protein kinase A-dependent phosphorylation and inhibition of glycine receptors that contain α3 subunits (α3 glycine receptors) [14, 27–29]. In lamina I of the spinal cord, peripheral neuropathy induces a down-regulation of the cell membrane potassium and chloride exporter KCC2 leading to an intracellular chloride accumulation and a subsequent decrease in the inhibitory effect of GABAergic and glycineric input [23]. In addition, several presynaptic mechanisms have been demonstrated. Epigenetic down-regulation of glutamate decarboxylase 65 (GAD65), which is one of the two GABA producing enzymes, reduces GABAergic inhibition [25] and peripheral nerve damage has been reported to induce apoptosis of GABAergic interneurons of the dorsal horn [30, 31]. A recent study demonstrated in addition reduced glycineric but unchanged GABAergic innervation of a certain type of excitatory dorsal horn neurons (radial cells) after peripheral nerve damage [32].

These findings suggest that restoring proper central pain control normally provided by GABAergic and glycineric neurons should be a highly rational approach to the treatment of chronic pain syndromes. Work with subtype-selective GABA<sub>A</sub> receptor modulators has shown that this strategy is effective at least in preclinical models of chronic pain [29, 33–35]. Similar evidence for selective pharmacological modulators of glycineric neurotransmission is still sparse but, given the strong glycineric innervation of the superficial dorsal horn and its known role in regulation of inflammatory pain, it appears likely that glycineric modulators should have similar or even stronger effects. With respect to side effects of pharmacological intervention, the glycineric system may have substantial advantages over that of GABA, due to its profound caudo-rostral gradient with strong innervation of the spinal cord and hindbrain and weaker innervation of the midbrain and forebrain [10]. In the forebrain, glycine receptors and glycineric axon terminals are much less abundant than GABA<sub>A</sub> receptors and GABAergic terminals [10]. The relative sparsity of glycineric forebrain innervation may help avoiding possible central side effects that typically limit the use of GABA<sub>A</sub> receptor modulators for many indications. Furthermore, about 75% of the inhibitory input onto excitatory neurons of the superficial dorsal horn is glycineric and only 25% GABAergic [8]. So, a certain degree of potentiation of glycine receptors will, in
comparison, lead to a much larger increase in central inhibition, when compared to that of GABAA receptors. Finally, since glycine and GABA are very often co-released from the same presynaptic terminals, a potentiation of glycine receptor function should restore inhibition even if the inhibitory loss occurred specifically for GABAA receptors.

Molecular targets within the dorsal horn glycinergic system

Enhanced efficacy of glycinergic inhibition may be achieved through compounds that target glycine receptors to enhance their activity or glycine transporters to interfere with glycine re-uptake (Fig. 3).

Glycine receptors

Glycine receptors are pentameric anion-permeable ion channels. Together with GABAergic, nicotinic acetylcholine and 5-HT3 receptors, they form the cys-loop superfamily of ion channels. The human genome harbors four genes (GLRA1, GLRA2, GLRA3, GLRB) that encode for the glycine receptor subunits α1, α2, α3, and β. The genomes of other mammals, including rodents and non-human primates, contain a fourth gene (glra4) encoding the α4 subunit. In humans, functional subunits cannot be expressed from the GLRA4 gene because of several amino acid exchanges and a premature STOP codon [36]. Functional glycine receptors can either be composed only of α subunits (homomeric) or can be heteromeric receptors composed of α and β subunits, most likely in a 2:3 stoichiometry [37]. Most adult glycine receptors are heteropentameric complexes containing α1 and β subunits. The glycine receptor subunit α3 is also contained in adult glycine receptors but its distribution is not as wide-spread as that of α1. In the spinal cord, α3 expression is concentrated in the superficial dorsal horn layers [14]. The α2 subunit is most strongly expressed in the developing nervous system, but has also been shown to be present in
areas in which glycinergic innervation is very sparse in the adult such as the cerebral cortex [38]. In some brain areas, such as the striatum, α2 subunits remain expressed at rather high levels into adulthood [39–41]. Additional evidence suggests that α2 subunits play a role in cortical development [42–44] and mutations in GLRA2 have been linked to autism spectrum disorders [45]. Unlike the different α glycine receptor subunits, the β subunit alone cannot form functional glycine receptors. However, the β subunits are required for postsynaptic clustering of glycine receptors via their interaction with gephyrin [46, 47] and in addition contribute to the glycine binding site in heteromeric receptors [37]. Glycine receptors devoid of β subunits (homomeric glycine receptors) cannot interact with gephyrin and are thus not enriched at postsynaptic sites. At extrasynaptic and presynaptic sites, they may still be important as mediators of tonic inhibition and as enhancers of neurotransmitter release in different CNS areas [48, 49]. As discussed below in detail, some glycine receptor modulators target preferentially or exclusively homomeric glycine receptors and thus may regulate tonic inhibition and presynaptic transmitter release rather than postsynaptic inhibition. All three processes would serve the aim of restoring a proper inhibitory tone in the spinal dorsal horn.

Several recent studies provide good evidence that positive allosteric modulation of glycine receptors reduces hyperalgesia in rodent models of chronic pain [29, 50–53]. One glycine receptor subtype (α3 glycine receptors) has attracted particular interest as a potential target for pain therapy. In the spinal dorsal horn (and in the trigeminal nucleus caudalis), these α3 glycine receptors are highly enriched to the superficial layers, where nociceptive sensory afferents terminate [14]. Although glycine receptors are expressed on presynaptic terminals of central neurons, they are absent from primary sensory neurons and their terminals ([54, 55] but see also [56]). Primary afferent depolarization and presynaptic inhibition of nociceptive input are not mediated by glycine. Inhibition of dorsal horn α3 glycine receptors by spinally produced PGE2 is a key mechanism of the central component of inflammatory hyperalgesia. Mice lacking α3 glycine receptors are protected from the pronociceptive effects of spinally injected PGE2 and recover much faster than their wild-type littermate from peripheral inflammation induced...
hyperalgesia [14, 28]. Since prostaglandins are largely irrelevant for the induction and maintenance of neuropathic pain, this inhibition does not occur in neuropathic pain models. Mice lacking α3 glycine receptors are, therefore, not protected from hyperalgesia or allodynia arising from neuropathic conditions [14, 57]. However, this does not implicate that α3 glycine receptors are irrelevant for neuropathic pain. It only indicates that the PGE2-mediated inhibition of α3 glycine receptors is not a relevant mechanism of sensitization in neuropathic pain (see also [29, 51, 53]).

Glycine transporters

Glycine is taken up from the extracellular space by two distinct cell membrane transporter proteins; GlyT1 (SLC6A9) and GlyT2 (SLC6A5). GlyT1 is widely expressed throughout the brain and spinal cord. It is mainly found in astrocytes [58–60] but has also been reported in neurons [61–63]. At glycineric synapses, GlyT1 plays a major role in the removal of glycine from the synaptic cleft following its release from presynaptic terminals. Mice lacking GlyT1 exhibit a hyperglycinergic phenotype with reduced muscle tone and respiratory deficits [64]. These mice are perinatal lethal. The expression of GlyT1 is, however, not limited to sites of glycineric innervation. GlyT1 is also densely expressed in the forebrain where it may regulate NMDA receptor function by controlling the glycine concentration at their glycine binding site (also known as glycineB site). Glycine transport by GlyT1 may reverse direction under certain conditions to promote calcium-independent glycine release from astrocytes [66]. Triggered by the idea that inhibition by GlyT1 might correct a deficit in NMDA receptor activity that underlies psychotic symptoms in schizophrenic patients, GlyT1 has attracted significant interest as a possible target for the development of antipsychotic (anti-schizophrenic) medications [67] but results of clinical trials were rather disappointing [68, 69].

GlyT2 is exclusively expressed by glycineric neurons, where it serves two functions. It (1) transports glycine from the synaptic cleft back into the cytoplasm of glycineric neurons to terminate the synaptic signal and (2) accumulates glycine to the level required for loading of presynaptic vesicles via the vesicular GABA transporter (vGAT), also known as vesicular inhibitory amino acid transporter (VIAAT) [70, 71]. Expression of GlyT2 is an indispensible prerequisite for a glycineric neuronal phenotype. Mice lacking GlyT2 display a hypoglycinergic phenotype with spasticity, tremor, and an inability to right [72]. They die roughly at 2 weeks of age from neuromotor deficiency. However, acute or incomplete block of GlyT2 delays the decay of glycineric postsynaptic currents via the extended presence of glycine in the synaptic cleft [73–75] and promotes tonic glycinergic currents [73, 75, 76]. Data from different laboratories strongly suggest an analgesic effect of both GlyT1 [77–80] and GlyT2 [77, 78, 80–87] inhibitors (for three recent reviews see refs. [88–90]).

What potential side effects need to be considered?

Glycine receptor modulators

Potential target-related (“on-target”) side effects may in principle originate from all processes that are regulated by glycine receptors. Obvious functions include, besides somatosensory processing, control of muscle strength, motor coordination, respiratory control, visual perception, hearing, and olfaction. Some evidence also points to a role of glycine in midbrain reward circuits (Fig. 4). Such potential side effects of glycine receptor activators/modulators have not yet been systematically assessed in pharmacological experiments. Most data discussed here are from morphological or electrophysiological experiments or report the phenotypes of knock-out mice. Below, we discuss potential side effects arising from CNS areas with significant glycineric innervation and whether selective targeting of glycine receptor subtypes might help reducing unwanted drug effects.

![Fig. 4 Glycinergic functions in the CNS that may potentially cause undesired drug effects. Potential addictive properties may arise from glycine receptors located in the Nucleus accumbens or the ventral tegmental area (VTA) where enhanced glycineric inhibition may lead to a disinhibition of dopamine release. Central respiratory rhythms depend on a well-balanced glycineric inhibition in the pre-Bötzinger complex. Glycine receptors are also found in parts of the CNS involved in primary sensory processing such as the retina, the olfactory bulb and in cochlear synapses. Potentiation of glycineric inhibition of motoneurons reduces muscle tone and may interfere with muscle strength. It should be kept in mind that the presence of glycine receptors in areas outside the pain system may also hint at potential additional indications such as impaired hearing or impaired respiratory drive](image-url)
Muscle tone

Glycine exerts a powerful feed-back and feed-forward control over motoneuron activity. Convulsions are the most obvious symptom of glycine receptor blockade by strychnine. Motoneurons express α1 and β glycine receptor subunits but lack α3 glycine [14]. Given the extremely weak muscle tone observed in GlyT1-deficient mice [64], it is likely that positive modulators of α1/β glycine receptors will cause muscle relaxation at sufficiently high doses. However, this does not necessarily mean that muscle relaxation will be a limiting side effect, as studies with GlyT1 inhibitors have found antinoceptive effects in mice at doses that did not cause movement problems [81]. Along the same lines, muscle relaxation by benzodiazepines, which also potentiate inhibitory input onto motoneurons [35, 91], is not a relevant concern as long as the patients’ muscle strength is not otherwise compromised. In fact, a certain degree of muscle relaxation might actually be beneficial in pain patients especially when pain conditions are accompanied by heightened muscle tone such as in low back pain. If muscle weakness turns out to be a problem of non-selective glycine receptor modulators, compounds devoid of activity at α1 (and α1/β) subtypes might offer a therapeutic solution.

Respiration

Impaired respiration is of major concern in opioid use and abuse. The acute life-threatening toxicity of opioids is due to respiratory depression. Respiratory failure can occur as a result of impaired neuronal breathing drive (as in opioids) or come from impaired neuromuscular function. Although not fully excluded, the latter is rather unlikely to occur with glycine receptor modulators. Impaired central respiratory control is, however, a relevant concern. Several lines of evidence indicate that well-balanced glycineergic activity is critical for respiratory rhythm generation. Mice lacking GlyT1 show highly reduced and irregular breathing activity and die within the first day after birth. Blockade of glycine receptors with strychnine injected into the pre-Bötzinger complex severely reduced respiratory activity [92]. Optogenetic stimulation of glycineergic neurons of the pre-Bötzinger complex prematurely terminated inspiratory activity and prolonged the interval until the next inspiratory phase [93]. Babies born with genetic defects in the glycineergic system have an increased risk of sudden infant death [94, 95]. These data are further supported by the expression of α1, α2, and α3 glycine receptors on neurons of the pre-Bötzinger complex, a key area of central respiratory control [96, 97]. Two studies have addressed the relative contribution of α1 and α3 glycine receptors to respiratory control in α1 and α3 subunit deficient mice. Mice lacking the glycine receptor α3 subunit survive and show no obvious deficits [14]. However, more-in-depth analyses have revealed subtle respiratory abnormalities such as a rather variable post-inspiratory phase [96]. The glycine receptor mutant mouse oscillator, which carries a frame-shift mutation in the glra1 gene preventing the expression of a functional α1 subunit protein, dies at about 3 weeks after birth [98] and exhibits greater respiratory abnormalities [99]. Taking these studies together, it appears that both increased and decreased glycineergic inhibition in the pre-Bötzinger complex compromise respiratory activity. Whether this poses a risk to the use of drugs enhancing glycineergic inhibition remains to be determined. In case of benzodiazepines, which are also linked to a certain degree of respiratory depression, therapeutic use is not curtailed unless used in combination with other sedative drugs or in patients with otherwise impaired respiratory drive. Furthermore, under conditions of impaired respiratory drive, potentiation of glycine receptors in the pre-Boetzing complex might also be beneficial [96].

Reward system

Addiction is a major concern severely limiting the use of opioid analgesic in pain patients with chronic non-malignant pain. Addiction is also a significant problem of chronic benzodiazepine use indicating that drugs that enhance inhibition (in this case via GABAA receptor modulation) can have a significant addictive potential [100]. A common denominator of most, if not all, drugs with liability to addiction is their ability to trigger dopamine release in the brain’s reward system, where drugs such as morphine excite (or more precisely disinhibit) dopaminergic neurons in the ventral tegmental area (VTA) that project to the Nucleus accumbens (NAc) [101]. Significant evidence suggests that glycineergic signaling in the VTA or NAc is involved in reward and addiction circuits. Glycine infused into the NAc causes dopamine release via its interaction with strychnine-sensitive glycine receptors [102]. Acute blockade of GlyT1 with Org 25935 inhibits ethanol intake and preference in rats [103]. At a first glance, it appears surprising that an inhibitory neurotransmitter increases dopamine release. However, a disinhibitory circuit in which enhanced glycineergic inhibition of GABAergic neurons relieves dopamine release from GABAergic inhibition is a likely explanation. A disinhibitory circuit also explains benzodiazepine-induced dopamine release from VTA neurons [104]. Dopamine release triggered by a number of addictive drugs (ethanol, tetrahydrocannabinol (THC) and nicotine [105]) is blocked by strychnine suggesting that glycine receptors are an element of the reward circuit of these drugs. In case of ethanol, this process probably involves the direct potentiation of glycine receptors by ethanol. Such a potentiation has been demonstrated not only in heterologous expression systems [106, 107], but also in acutely dissociated VTA neurons whose glycine receptors
are potentiated by ethanol concentrations reached in the brain after “social” drinking (10–20 mM; [108]) [109]. Moreover, a recent report has shown that medium spiny neurons of the VTA, which are GABAergic and inhibit dopamine release, express ethanol-sensitive α1 glycine receptors at high density [110]. These data strongly support the concept of a disinhibitory action of ethanol and glycine receptors in the VTA/NAc reward circuits.

Drugs that activate and/or potentiate the action of glycine may thus have rewarding properties and may carry a risk for addiction. Even if this turns out to be the case, it might still be possible to avoid addiction by targeting specific glycine receptor subtypes. Work on the mechanisms of benzodiazepine addiction has shown that their addictive properties can be avoided with compounds that lack efficacy at α1 GABA_A receptors (such as L-838'417) [104]. With the presently available data, a potential liability to addiction cannot be attributed to a specific glycine receptor subtype. All glycine receptor α subunits (α1, α2, and α3) are expressed in the NAc and VTA [40, 41]. A recent study that compared ethanol effects in wild-type and α2 or α3 glycine receptor deficient mice found that rewarding properties were more strongly affected by deletion of α2 than of α3 glycine receptors [111]. α3 glycine receptors even appeared to have an anti-ethanol seeking effect. On the other hand, a genetic association study in African Americans found a statistically significant link between alcoholism and single nucleotide polymorphisms (SNPs) in the GLRA3 gene [112]. It is at present not known whether these SNPs are accompanied by increased or decreased glycinergic activity. In any case, drug discovery programs should carefully evaluate potential liability to addiction of glycine receptor modulators.

**Vision, hearing and olfaction**

Glycine receptors are also expressed in the retina, the auditory and olfactory systems. Studies in glycine receptor knock-out mice have found phenotypes in tests of acoustic or visual function [113–115]. Whether modulators of glycine receptor would affect sensory function is currently unknown. Early work in humans from the 1960s reported minor effects of non-convulsive doses of strychnine on sensory systems with small and statistically insignificant increases in visual acuity and speech perception [116, 117]. On the other hand, some recent evidence points to a potential use of glycine receptor modulators in patients with tinnitus [118, 119] or impaired hearing [115].

**Potential excitatory effects of glycine in neuropathic conditions**

A potentiation of glycine receptor activation can compensate for diminished inhibition caused by partial glycine receptor blockade or diminished glycine release. However, in cases when a down-regulation of the potassium chloride exporter KCC2 increases intracellular chloride concentration and turns glycinergic inhibition into excitation, facilitation of glycinergic input may actually promote pain [23]. Several lines of evidence indicate that drugs which increase GABAergic or glycinergic spinal inhibition do not enhance nociceptive responses even after peripheral nerve damage, the condition for which a down-regulation of KCC2 has been demonstrated [23]. First, spinally applied benzodiazepines reduce both inflammatory and neuropathic hyperalgesia in mice, although experiments with GABA_A receptor point mutated mice have unambiguously shown that this is a genuine antihyperalgesic effect occurring through a facilitation of GABA_A receptors in the spinal dorsal horn [33, 35, 120]. Chemogenetic activation of glycinergic dorsal horn neurons reduces not only acute pain responses but also nerve injury-induced hyperalgesia [8]. Finally, experiments with several positive allosteric glycine receptor modulators (DH-CBD, LT-01-25, 2,6-DTBP, AM-1488, for details see Table 1) revealed clear antihyperalgesic effects in a rodent model of neuropathic pain [29, 51–53]. That does, however, not exclude that a disturbed chloride homeostasis would limit analgesic effects of glycine or GABA_A receptor potentiators. The increase in chloride influx caused by glycine receptor modulators may overcharge the neurons’ chloride extrusion system, which is compromised in neuropathic pain conditions [23]. Down-regulation of KCC2 may hence potentially reduce the therapeutic efficacy of glycine receptor modulators. Combinations of glycine receptor modulators with compounds that can correct impaired chloride homeostasis such as KCC2 modulators or carboxyhydrate inhibitors might be an interesting and complementary option [121].

**Potential side effects of glycine transport modulation**

Both data obtained from GlyT1-deficient mice [64] and with GlyT1 inhibitors [122] demonstrate that GlyT1 plays a dominant role in the removal of glycine from the synaptic cleft. In addition, GlyT1 may limit the activity of extrasynaptic glycine receptors and thereby regulate tonic glycinergic inhibition. Blocking GlyT1 should, therefore, allow a fine-tuned enhancement of neuronal inhibition. Problems may, however, arise from increases in local glycine in the vicinity of NMDA receptors which require the binding of glycine (or D-serine) to the so called glycine B site for full activation [123]. Enhanced NMDA receptor activation by increased glycine concentrations may be particularly relevant in the spinal cord where spill-over of synaptically released glycine to neighboring NMDA receptors has been demonstrated [124]. Because NMDA receptor activation is typically linked to pain perception and the generation of hyperalgesia, this effect may counteract the analgesic effect of increased
Table 1  GlyR modulators

<table>
<thead>
<tr>
<th>Modulator</th>
<th>Group</th>
<th>Potency, μM (EC₅₀)</th>
<th>GlyR selectivity</th>
<th>Techniques employed</th>
<th>Additional targets</th>
<th>Pain models</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cannabidiol (CBD), dehydroxyl-cannabidiol (DH-CBD)</td>
<td>Cannabinoid ligand</td>
<td>≈3–5</td>
<td>α₁ ≈ α₃ &gt; α₂</td>
<td>Electrophysiology</td>
<td>GPR55, CB2, TRPV1, PLA2, 5-HT₁A</td>
<td>CFA; spinal nerve ligation</td>
<td>[50, 169, 170]</td>
</tr>
<tr>
<td>2,6-Di-tert-butylphenol (2,6-DTBP)</td>
<td>Propofol analog</td>
<td>≈20–25</td>
<td>α₃ &gt; α₁</td>
<td>Electrophysiology</td>
<td>HCN1</td>
<td>Zymosan A, CFA; chronic constriction injury</td>
<td>[29, 137]</td>
</tr>
<tr>
<td>Gelsemine</td>
<td>Natural alkaloid</td>
<td>≈0.6–49</td>
<td>α₂ ≈ α₃ (inhibition) α₁ (biphasic modulation)</td>
<td>Electrophysiology, Ê-H-strychnine binding</td>
<td>ND</td>
<td>Formalin-induced pain, bone cancer, spinal nerve ligation</td>
<td>[156, 157]</td>
</tr>
<tr>
<td>AM-1488/AM-3607</td>
<td>Tricyclic sulfonamide</td>
<td>≈0.016–0.066</td>
<td>α₁ ≈ α₂ ≈ α₃</td>
<td>Fluorescence-based membrane potential assays</td>
<td>ND</td>
<td>Spared nerve injury</td>
<td>[53, 163]</td>
</tr>
<tr>
<td>Compound 19 and (ent2)-20</td>
<td>Azetidine sulfonamides</td>
<td>0.7 (at α₃/β)</td>
<td>α₁ = α₃</td>
<td>Fluorescence-based chloride flux measurement</td>
<td>ND</td>
<td>ND</td>
<td>[164, 171]</td>
</tr>
<tr>
<td>Compound 2 (4-fluoro-N-(2-(quinolin-8-yloxy)ethyl) benzenesulfonamide)</td>
<td>Sulfonamide</td>
<td>≥3.9–8.0</td>
<td>α₁ = α₃</td>
<td>Fluorescence-based chloride flux measurement</td>
<td>ND</td>
<td>ND</td>
<td>[162]</td>
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<tr>
<td>Compounds 3 and 6</td>
<td>sesterterpene glycinyl-lactams</td>
<td>≥8.5 (compound 3)</td>
<td>Compound 3: α₁ &gt; α₃ Compound 6: α₃ &gt; α₁</td>
<td>Electrophysiology</td>
<td>ND</td>
<td>ND</td>
<td>[159–161]</td>
</tr>
<tr>
<td>Tropisetron (ICS-205,930)</td>
<td>tropeines</td>
<td>nM &gt; 10 μM</td>
<td>α₁/β, α₂/β</td>
<td>Electrophysiology</td>
<td>5-HT₃</td>
<td>5-HT₃</td>
<td>[144]</td>
</tr>
</tbody>
</table>

ND not determined, CFA complete Freund's adjuvant

*Biphasic modulation
activation of inhibitory glycine receptors [77, 79]. NMDA receptor-related side effects might also occur at other sites in particular in the forebrain, which is rich in GlyT1 protein and NMDA receptors. In these brain areas, which are basically devoid of glycinergic innervation, GlyT1 still regulates NMDA receptor activity by controlling the concentration of ambient extracellular glycine at the glycine B site [125, 126]. The role of GlyT1 in this process has actually made GlyT1 blockers candidates for anti-schizophrenic treatment. Some recently developed GlyT1 inhibitors have been promoted to clinical trials in schizophrenic patients. These clinical trials yielded disappointing results with respect to improvement of psychotic symptoms [68, 69] but severe side effects were not reported. Whether these compounds possess analgesic efficacy in human patients with chronic pain is currently unknown.

Side effects observed in preclinical tests with sarcosinelike irreversible GlyT1 inhibitors (NFPS, ORG 25935 and RG1678) were partially reminiscent of the phenotypes of GlyT1-deficient mice and related to over-stimulation of inhibitory glycine receptors. Such problems might not occur with more recently developed competitive inhibitors, as their effects would be self-limiting. This concept may be helpful when increased activation of NMDA receptors is the desired effect (such as in the schizophrenia indication). However, when inhibitory glycinergic transmission shall be enhanced with competitive GlyT1 blockers, nearly full activation of the glycine B site at NMDA receptors will occur with potential negative consequences on hyperalgesia. An alternative and, in our opinion more promising, approach might be the development of reversible allosteric GlyT1 inhibitors that cause only incomplete blockade even at saturating concentrations. Competitive inhibitors may also offer opportunities, if they have low affinities (to prevent saturation of NMDA receptors) but slow dissociation kinetics to prevent fast displacement by synaptically released glycine.

GlyT2 inhibitors are less likely to cause NMDA receptor-related side effects although it cannot be completely excluded that reduced reuptake of glycine via GlyT2 at sites with glycinergic innervation also fosters glycine spill-over to NMDA receptors. Problems might, however, arise from the dual role of GlyT2 at glycinergic synapses. The primary function of GlyT2 is very likely pro-glycinergic by providing glycine to presynaptic glycinergic terminals for synaptic release. Blocking GlyT2 function will, therefore, have to be closely balanced to yield the desired prolongation of glycine presence in synapses without reducing glycine filling of presynaptic glycinergic terminals. Org 25543, one of the earliest GlyT2 inhibitors, indeed prolongs glycinergic IPSCs after acute application in spinal cord slices but glycinergic synaptic events become greatly diminished after prolonged exposure [73]. It acts as a nearly irreversible inhibitor and induces lethal convulsions at doses only ten-times higher than those required for half-maximal analgesia (suppression of flinches in the formalin test) and still below full target (GlyT2) exposure [85]. These side effects are reminiscent of the phenotype of GlyT2-deficient mice and likely caused by a deficit in glycinergic inhibition. Such side effects may be avoided by more recently developed competitive (reversible) inhibitors (compound 1 in [85]), which did not induce convulsions after acute treatment. However, sedation was observed with this compound at high doses.

Drug-like modulators of glycine receptors

Ligands with modulator activity at inhibitory glycine receptors but with low selectivity have been known for longer. Such compounds include bivalent cations, mono- and disaccharides, glutamic acid, ginkgolic acids, ivermectins, synthetic neurosteroids, and n-alcohols. Their actions on glycine receptors have been reviewed previously (e.g. [127, 128]). Given the presumed therapeutic potential of glycine receptor modulators, a need for molecules with drug-like properties was evident. Here, we focus on glycine receptor modulators that have already been tested in pain models and on molecules, which have been discovered recently through unbiased high throughput screen (HTS) of large libraries of synthetic or natural compound libraries. Table 1 provides an overview about these compounds and Fig. 5 illustrates the current knowledge about their respective binding sites on the mammalian crystal structure of glycine receptors.

Derivatives of existing drugs

Propofol derivatives

The intravenous anesthetic propofol (2,6-diisopropylphenol) primarily targets GABA_A receptors but also modulates glycine receptors [129, 130]. Due to a ~tenfold lower potency at glycine receptors relative to GABA_A receptors, it is unlikely that these receptors contribute significantly to propofol anesthesia ([131, 132], see, however [133]), but halogenated propofol derivatives (4-fluoro propofol, 4-bromo propofol, and 4-chloropropofol) have increased potencies at glycine receptors [134, 135] reaching values comparable to those at α1β3γ2 GABA_A receptors [136]. A large series of propofol derivatives has been synthesized in an attempt to correlate the anesthetic properties of propofol with its GABA_A receptor modulatory effects [137]. Among these compounds was 2,6-DTBP which is completely devoid of anesthetic actions and lacks modulatory and agonistic activity at α1β3γ2 GABA_A receptors. Subsequent work verified the absence of modulatory properties at GABA_A receptors [138] but demonstrated activity at glycine receptors [139]. The actions of 2,6-DTBP on glycine receptors
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and on hyperalgesia have been extensively investigated in a recent study [29]. 2,6-DTBP turned out to be an allosteric modulator of recombinant homomeric α1 and α3 glycine receptors with no activity at recombinant and native spinal cord GABA<sub>A</sub> receptors. Critical for its interaction with glycine receptors is a phenylalanine residue (F388, in α3) in the so-called membrane-associated stretch of the intracellular loop between transmembrane segment TM3 and TM4. This residue is also critical for the interaction of the channel with propofol. Interestingly, recombinant heteromeric α3β and synaptic glycine receptors were potentiated only in a phosphorylated configuration. The relevant phosphorylation site is serine 346, which becomes phosphorylated in vivo in inflammatory pain conditions and in response to PGE2 [14], a pivotal inflammatory mediator which is produced in the spinal cord in response to peripheral inflammation [140]. Posttranslational modifications such as phosphorylation may add another level of specificity to glycine receptor modulation. Consistent with this dependence on phosphorylation, 2,6-DTBP exerted analgesic effects only in models of chronic pain. Although 2,6-DTBP had no activity at GABA<sub>A</sub> receptors, it still targets other ion channels including HCN1 channels [141]. This lack of general specificity and the high doses (90 mg/kg, i.p.) required for in vivo effects make 2,6-DTBP a poor drug candidate. However, a patent search revealed new propofol derivatives (such as 4-[(benzylxoy)-3,5-diisopropylbenzoyl chloride; LT-01-25) with potency for glycine receptor modulation in the subnanomolar range [52]. In rodent experiments, LT-01-25 normalized mechanical and cold allodynia in the streptozotocin model of painful diabetic neuropathy at doses of 10–20 mg/kg p.o.

**Tropeines**

Tropeines are potent 5-HT<sub>3</sub> receptor antagonists. They are clinically used as anti-emetic drugs in patients with chemotherapy induced nausea and vomiting. Tropisetron, also known as ICS-205,930, is one of the best known members of this group. Two tropeines, MDL-72222 and tropisetron, in addition potentiate glycine receptor currents in nanomolar concentrations, while higher micromolar concentrations cause inhibition [142]. Tropeines appear to bind to an interface formed by two α or one α and one β subunit in the extracellular domain of glycine receptors close to the glycine binding site [143–146]. Tropisetron displays some subunit-specificity with potentiation of homomeric α1 but inhibition of homomeric α2 glycine receptors. Co-expression of β subunits significantly increased the sensitivity of α1 glycine receptors to potentiation and turned inhibition of α2 glycine receptors into potentiation [144]. The high affinity binding and the remarkable sensitivity of GlyRs to tropeines makes this group of compounds one of the most promising candidates for the development of specific drugs targeting glycine receptors. However, despite of some differences between the chemical requirement for tropeine binding to glycine and 5-HT<sub>3</sub> receptors, most tropeines still bind and modulate 5-HT<sub>3</sub> receptors with high affinity [145].

Fig. 5 Modulatory sites at glycine receptors. The most relevant amino acids (highlighted in colored spheres) are shown in a single α3 subunit (right) and for a homopentameric α3 glycine receptor (left). The structural data (PDB ID:5TIO) were taken from [53]. For the compound AM-3607, which binds to an interface of two adjacent subunits, only the residues of the (+) subunit are shown [53]. The residues relevant for the actions of tropeines on glycine receptors have not yet been identified for α3 glycine receptors. The sites indicated are the homologous sites identified originally for the α1 glycine receptor subunit [143–146]. The sites for THC, CBD, propofol and 2,6-DTBP were identified and characterized in [29, 30, 148, 168]. Glycine molecules bound to the orthosteric sites are shown in green.
understanding of the structural basis of the interaction of glycine receptor subtypes with tropeines may perhaps lead to new tropeine derivatives acting as specific enhancers of glycineric inhibition.

Natural ligands

Plant-derived cannabinoids

Although G protein-coupled CB1 and CB2 receptors constitute the main targets of cannabinoids, several of these molecules, Δ9-tetrahydrocannabinol (THC), cannabidiol (CBD) and its synthetic derivative dehydroxyl-cannabidiol (DH-CBD), also modulate glycine receptors [50, 51, 147–149].

THC was the first phytocannabinoid to be shown to potentiate glycine receptor responses [147]. In an effort to produce derivatives that more specifically interact with glycine receptors, 5-desoxy THC (identical to dehydroxyl-cannabidiol, DH-CBD), 1-desoxy THC and di-desoxy THC (identical to di-dehydroxyl-cannabidiol, DD-CBD) were generated [50]. While THC exerts effects on both G protein-coupled CB1 and CB2 receptors and on glycine receptors, DH-CBD has strongly reduced affinity to CB1 and CB2 receptors but still potentiates glycine receptors with unaltered potency and efficacy. DD-CBD does no longer bind to CB1 receptors and is an antagonist at the THC binding site of glycine receptors.

In case of CBD and DH-CBD, potentiation occurs through an increase in the apparent affinity for glycine [51, 150]. THC exhibits some preferential activity at α1 and α3 glycine receptors, which are potentiated by THC with similar potency and efficacy, while α2 glycine receptors exhibit a significantly lower sensitivity [50]. The differences between subunits allowed the identification of structural requirements of glycine receptors for modulation by THC. Sequence comparisons of the highly sensitive α1 and α3 with low sensitivity α2 glycine receptors suggested that a conserved serine residue within the transmembrane domain 3 (TM3) (i.e. S296 in α1 and S307 in α3GlyR) confers high sensitivity to THC [50]. Substitution of this amino acid with the corresponding alanine residue present in α2 glycine receptors (A302) significantly decreased the sensitivity of α1 and α3 glycine receptors. Subsequent NMR analyses with purified transmembrane glycine receptor domains together with molecular modeling revealed that these serine residues are directly interacting with THC and DH-CBD [50, 148].

The action of glycine receptor modulators is in several cases different for homomeric and α/β heteromeric receptors. This has been specifically shown for 2,6-DTBP [29] and applies also to DH-CBD/5-desoxy THC [50, 149]. Such a difference may be relevant for potential in vivo applications because glycine receptors at postsynaptic sites are α/β heteromeric receptors [151–153], while presynaptic glycine receptors are considered a homomeric receptors. Specific modulation of homomeric or heteromeric receptors may thus restrict activity to presynaptic or postsynaptic sites and may have specific therapeutic indications [149].

Consistent with this in vivo glycine receptor modulation THC, CBD and DH-CBD reduce hyperalgesia in inflammatory and/or neuropathic rodent pain models [50, 51]. Analogic actions of THC in acute pain models are well established and part of the classical tetrad of in vivo CB1 receptor-mediated effects. Acute analogic effects of THC were accordingly blocked by a CB1 receptor antagonist (AM-251) and absent in CB1 receptor (CB1−/−) deficient mice. However, the antihyperalgesic effects of THC in chronic pain models persisted in CB1−/− mice and were blocked by di-desoxy THC/DD-CBD [50]. A subsequent study demonstrated that DH-CBD also exerts antihyperalgesic effects that were blocked by di-desoxy THC/DD-CBD [51]. Interestingly, the antihyperalgesic actions of all three compounds (THC, DH-CBD and CBD) depended on the expression of α3 glycine receptors [50, 51]. These encouraging results together with the availability of structural information from crystalized α1 glycine receptors have stimulated virtual screening efforts which have identified 12 previously unknown glycine receptor modulators among 1549 FDA approved drugs [154]. These compounds have not yet been tested in vivo for potential analgesic efficacy, but their discovery may foster such studies in the future.

Gelsemine-derived alkaloids

The alkaloid gelsemine has been recently shown to have activity at glycine receptors. Gelsemine is one of the principal alkaloids produced by the Gelsemium genus plants [155]. Gelsemine elicits anti-nociceptive effects in rat models of acute chemical and neuropathic pain after intrathecal injection but not after local peripheral or intracerebroventricular injection [156]. The antineuropathic pain effects were blocked by strychnine and after si-RNA mediated knockdown of spinal α3 glycine receptors but not of α1 glycine receptors. Subsequent characterization of gelsemine action on glycine receptor function revealed rather complex effects [157]. The authors observed a bi-phasic action on homomeric α1 glycine receptors with potentiation at low concentrations and inhibition at higher concentrations, while all other glycine receptors (α1/β heteromeric receptors, and α2 and α3 homomeric and α2/β and α3/β heteromeric receptors) were inhibited. Native glycine (and GABA_A) receptors in cultured spinal cord neurons were inhibited by gelsemine. In spinal cord slices, the frequencies of spontaneous glycine and glutamate mediated mIPSCs and mEPSC_S were reduced while amplitudes were not affected. It remains at present unclear how these complex cellular effects relate to the in vivo antinociceptive effects of gelsemine.
New lead structures revealed based on molecules obtained from marine sponges

The screening of natural compound libraries is a highly timely approach to the discovery of new chemical entities acting on an identified target. Libraries obtained from marine organisms appear to be particularly rich in hitherto unknown biologically active molecules [158]. One such screen at the University of Queensland, Australia, was performed on a library of more than 2500 compounds extracted from southern Australian and Antarctic marine invertebrates and algae. It led to the identification of three Irciniidae sponges that contained sesterterpene glycinyln-lactam compounds with activity at glycine receptors [159–161]. Following an initial screen based on a fluorescent chloride sensitive protein, detailed chemical characterization and electrophysiological analyses identified two novel glycine receptor modulators from Psammocinia sponges (a genus of the Irciniidae family) with considerable specificity for either α1 or α3 glycine receptors. Compound 3 in ref. [160] specifically potentiated responses in α3 glycine receptors with virtually absent activity in α1 glycine receptors, and compound 6 potentiated α1 glycine receptors while moderately blocking α3 glycine receptors. Potential effects of these compounds on native receptors or in vivo actions have not been reported, likely because due to the highly complex structure of both compounds chemical synthesis of larger quantities is a major challenge. Nevertheless, the discovery of the glycinyln-lactam structure as a new pharmacophore for subtype-specific glycine receptor modulation should constitute an interesting starting point for new drug discovery programs.

New chemical scaffolds

Up until recently, published glycine receptor modulators suffered greatly from low potency and insufficient selectivity over other cys-loop channel subtypes and/or unfavorable pharmacokinetic properties limited their suitability for in vivo studies. This triggered a large industry effort to identify chemical matter that would alleviate these limitations and address more specifically the desired therapeutic application. Drug companies have published some of their efforts on new glycine receptor modulators that were discovered from unbiased screens of large chemical libraries [53, 162–164].

The screen done by Neusentis (a former research unit of Pfizer) aimed specifically at positive modulators of α3 glycine receptors [162]. This group employed fluorescence membrane potential assays for a first selection, which yielded 147 hits from more than 55,000 compounds. Medium throughput electrophysiology verified four compounds and a subsequent additional membrane potential screen of 1986 compounds identified another 31 hits, with nine compounds verified in electrophysiology. Seven compounds were evaluated in manual patch-clamp experiments. 4-fluoro-N-(2-(quinolin-8-yl)oxy)ethyl)benzene sulfonamide (compound 2) showed an EC50 for positive allosteric modulation of α3 glycine receptors of around 5–6 μM. Although identified in a screen for the α3 glycine receptor subtype, it had also modulatory activity at α1 glycine receptors. It did not activate glycine receptors directly and did not elicit any functional effects at α1β3γ2 or α3β3γ2 GABA receptors. So far, no in vivo data have been published on these hits.

Amen [163] utilized a similar FLIPR-based HTS strategy targeting human α3β glycine receptors which resulted in the discovery of a novel class of tricyclic sulfonamides. These hits were further improved into high quality proof-of-concept and co-crystallization molecules further supporting the role of glycine receptors and their potential in being used as therapeutic analgesics. Indeed, Huang and co-workers [53, 163] demonstrated how a rather low potency first hit was subsequently optimized for potency and tissue penetration sufficient for ex vivo tests of the compound on native mouse dorsal horn glycine receptors. Further optimization led to a compound (AM-1488) that was metabolically stable enough for in vivo testing. At an oral dose of 20 mg/kg, AM-1488 reduced allodynia in mice with spared nerve injury-induced neuropathic pain to a degree similar to pregabalin (30 mg/kg). AM-1488 did not significantly impair movement in naïve mice and had no detectable off-target effects on other Cys-loop receptors or membrane proteins in in vitro screens suggesting that the observed anti-allodynia effect was due to its action on glycine receptors. This compound was then further optimized to yield the high potency/affinity compound AM-3607 [163]. Although the initial screen targeted α3 glycine receptors, both compounds enhanced the activity of homomeric and heteromeric α1 and α3 glycine receptors (EC50 in ranges from 0.016 to 0.066 μM). The affinity of AM-3607 to α3 glycine receptors was amenable for co-crystallization with human α3 glycine receptors. X-ray analyses at 2.6-Å resolution revealed a new inter-subunit allosteric binding site located in the extracellular domain and approximately 10 Å away from the orthosteric glycine binding site (Fig. 5). The close association of the AM-3607 site with the orthosteric site and further biochemical experiments is consistent with a stabilizing action of AM-3607 on glycine binding and a subsequent increases the apparent affinity for glycine and channel activity. This binding pocket is conserved between α1 and α3 subunits explaining why AM-1488 does not differentiate between α1 and α3 glycine receptors. More recently, Chakka et al. [164] reported two novel classes of glycine receptor potentiators using similarity- and property-guided scaffold hopping enabled by parallel synthesis and pharmacophore-based virtual screening strategies. These included azetidine sulfonamides and amnithiazole sulfone leads that have been reported to bind in

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the previously described AM-3607 glycine receptor pocket. These novel scaffolds combine potencies in the low micromolar and submicromolar range with selectivity profiles that favour glycine receptors over other cys-loop family receptors and with ADME-like properties. They therefore significantly expand the current tools available for the analysis of glycine receptor function. In combination with the recently solved crystal structure of a mammalian glycine receptor, they are also anticipated to foster the discovery and development of novel pain therapeutics.

Conclusions

Available data clearly support a pivotal role of glycine-ergic inhibition in spinal nociception. Positive allosteric modulation of glycine receptors and inhibition of glycine re-uptake appear as attractive opportunities to restore proper synaptic inhibition in spinal nociceptive circuits. Antinociceptive effects of glycine receptors modulators or glycine transport inhibitors have already been demonstrated in pre-clinical models of chronic pain. Open questions relate to the therapeutic value of this target in chronic pain in human patients and at least equally relevant to possible unwanted effects. GlyT1 inhibitors, which have already been in large-scale clinical trials for indications different from pain, are well tolerated. Much less is known in case of GlyT2 inhibitors and especially in the emerging field of glycine receptor modulators. It is likely that such compounds will be devoid of many side effects of centrally acting analgesics and of drugs that target the GABAergic system, but solid evidence is still missing. Future studies on drug candidates should carefully evaluate potential adverse effects arising from impaired muscle strength or respiratory drive. Potential liability addiction due to activation of glycine receptors in the brain’s reward circuit is another potential concern. Closely related to this question is whether therapeutically used glycine receptor modulators will need to show subunit-specificity (either α1 or α3). Subtype-specific modulators may be less prone to side effects but may also have lower analgesic efficacy. With last year’s arrival of first compounds suitable for in vivo preclinical testing, we will hopefully soon know more about the potential that this new target carries for the treatment of patients in chronic pain.

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