British Journal of Pharmacology

DOI:10.1111/j.1476-5381.2011.01471.x
www.brjpharmacol.org

REVIEW

Allosteric modulation of glycine receptors

Gonzalo E. Yevenes[1] and Hanns Ulrich Zeilhofer[1,2]

[1]*Institute of Pharmacology and Toxicology, University of Zurich, Zurich, Switzerland, and*
[2]*Institute of Pharmaceutical Sciences, ETH Zurich, Zurich, Switzerland*

Correspondence
Dr HU Zeilhofer, Institute of Pharmacology and Toxicology, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland.
E-mail: zeilhofer@pharma.uzh.ch

Keywords
allosteric modulation; cannabinoid; glycine; endocannabinoid; ethanol; glycine receptor; neurosteroids; tropeines; zinc

Received
7 March 2011
Revised
21 April 2011
Accepted
29 April 2011

Inhibitory (or strychnine sensitive) glycine receptors (GlyRs) are anion-selective transmitter-gated ion channels of the cys-loop superfamily, which includes among others also the inhibitory γ-aminobutyric acid receptors ($GABA_A$ receptors). While GABA mediates fast inhibitory neurotransmission throughout the CNS, the action of glycine as a fast inhibitory neurotransmitter is more restricted. This probably explains why $GABA_A$ receptors constitute a group of extremely successful drug targets in the treatment of a wide variety of CNS diseases, including anxiety, sleep disorders and epilepsy, while drugs specifically targeting GlyRs are virtually lacking. However, the spatially more restricted distribution of glycinergic inhibition may be advantageous in situations when a more localized enhancement of inhibition is sought. Inhibitory GlyRs are particularly relevant for the control of excitability in the mammalian spinal cord, brain stem and a few selected brain areas, such as the cerebellum and the retina. At these sites, GlyRs regulate important physiological functions, including respiratory rhythms, motor control, muscle tone and sensory as well as pain processing. In the hippocampus, RNA-edited high affinity extrasynaptic GlyRs may contribute to the pathology of temporal lobe epilepsy. Although specific modulators have not yet been identified, GlyRs still possess sites for allosteric modulation by a number of structurally diverse molecules, including alcohols, neurosteroids, cannabinoids, tropeines, general anaesthetics, certain neurotransmitters and cations. This review summarizes the present knowledge about this modulation and the molecular bases of the interactions involved.

Abbreviations

2-AG, 2-arachidonyl-glycerol; 3α,5α-THPROG, 3α,5α-tetrahydroprogesterone, allopregnanolone; 5β-pregnan-3α-ol-20-one, 3α,5β-THPROG, pregnanolone; AEA, N-arachidonoyl ethanol amide; CB receptor, cannabinoid receptor; GlyR, glycine receptor; NA-glycine, N-arachidonoyl glycine; TM, transmembrane

Introduction

Strychnine-sensitive glycine receptors (GlyRs) are pentameric anion channels. Five GlyR subunits have been cloned from mammalian tissue and designated α1–α4 and β (Lynch, 2004). Each GlyR subunit contains an amino-terminal extracellular domain, four transmembrane domains (TM) and a large intracellular loop between TM3 and TM4, which configure the ligand binding region, the ion channel pore and sites for intracellular modulation respectively (Lynch, 2004; Sine and Engel, 2006; Baenziger and Corringer, 2011).

In the adult, most GlyRs are composed of α1 and β subunits probably in a 2(α1)/3β stoichiometry (Grudzinska *et al.*, 2005). This GlyR subtype is the main mediator of glycinergic inhibition in the adult CNS. Many of these GlyRs colocalize with the postsynaptic scaffolding protein gephyrin (Todd *et al.*, 1995; Waldvogel *et al.*, 2010). Early in development most GlyRs are α2 homomers, which become replaced around postnatal day 14 by α1β heteromers in most CNS areas (Malosio *et al.*, 1991; Lynch, 2004). In a few selected areas, such as the retina, α2 persists however into adulthood. Like α1 subunits, α3 subunits are mainly found in the adult but their expression is spatially much more restricted. Immunohistochemistry and quantitative RT-PCR studies in mice have shown that α3-GlyR subunits are predominantly expressed in the superficial laminae of the dorsal horn

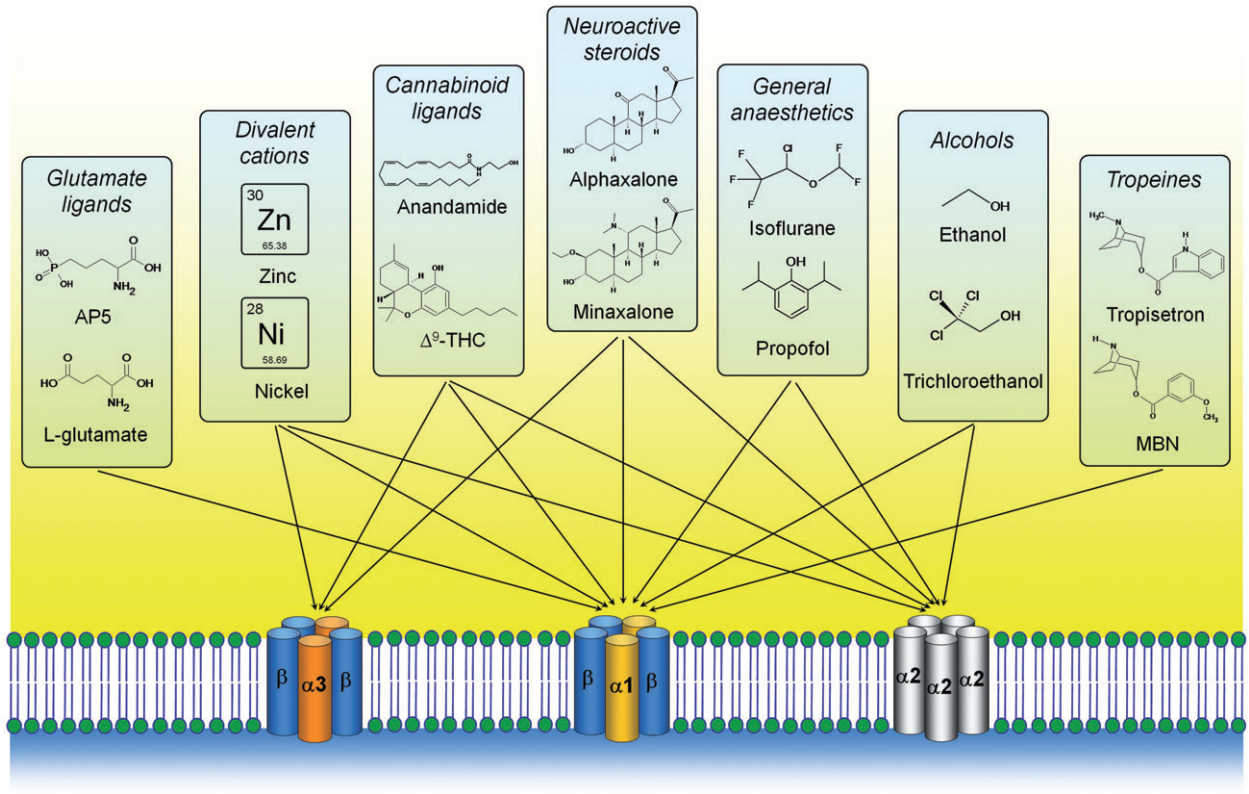

Figure 1

Allosteric modulation of GlyR subtypes. The scheme summarizes the interactions between several groups of allosteric modulators with different GlyR subtypes. Examples of some representative chemical structures for each group of compounds are shown.

(Harvey *et al.*, 2004; Anderson *et al.*, 2009) and in the respiratory network of the brainstem (Manzke *et al.*, 2010). RNA edited α2- and α3-GlyRs may serve a peculiar function as extrasynaptic high affinity GlyRs in the hippocampus (Meier *et al.*, 2005; Legendre *et al.*, 2009). The gene encoding for the GlyR α4 subunit is a pseudogene in humans due to the presence of a premature stop codon (Simon *et al.*, 2004).

The subunit composition of the ion channel complex and the arrangement of the different subunits within this complex determine its pharmacological profile. According to recent data, the glycine binding site of α/β heteromeric GlyRs is jointly formed by α and β subunits (Grudzinska *et al.*, 2005), while β subunits interact with gephyrin and thereby mediate synaptic clustering of GlyRs (Pfeiffer *et al.*, 1982; Schmitt *et al.*, 1987; Kim *et al.*, 2006). Although very few established drugs primarily act through inhibitory GlyRs (Laube *et al.*, 2002), a number of endogenous messenger molecules and some drugs do modulate GlyRs function (Figure 1). Such compounds include different cations, in particular zinc, cannabinoids, neuroactive steroids, tropeines, alcohols, avermectins, butyrolactones and general anaesthetics. Most of these molecules do not directly interact with the glycine binding sites but rather bind to allosteric sites within the GlyR complex (Figure 2). It is at present not yet established to what extent these interactions contribute to physiology or to drug actions *in vivo*, but their existence clearly establishes a possibility for specific pharmacological intervention. The analysis of the molecular bases of the interaction of these compounds with GlyRs should hence foster the development of specific GlyR modulators.

Possible indications of GlyR as a therapeutic target

Early knowledge about possible roles of glycinergic neurotransmission in physiological functions or in diseases has mainly been obtained through pharmacological blockade of GlyR with the rodent poison strychnine (for a review see Callister and Graham, 2010). These early studies have identified a critical role of GlyRs in the control of muscle tone. Severe cramps of the skeletal musculature are the leading symptom of strychnine poisoning. Apart from these motor symptoms altered sensory perception such as an increased sensitivity to acoustic or tactile stimuli has also frequently been observed. Further evidence for the involvement of glycinergic inhibition in sensory processing has come from studies in several strains of GlyR mutant mice (*spasmodic*, *oscillator* and *spastic*) which carry mutations in the GlyR α1 subunit (*spasmodic*, *oscillator*) or in the β subunit (*spastic*) (Buckwalter *et al.*, 1994; Mülhardt *et al.*, 1994; Ryan *et al.*,

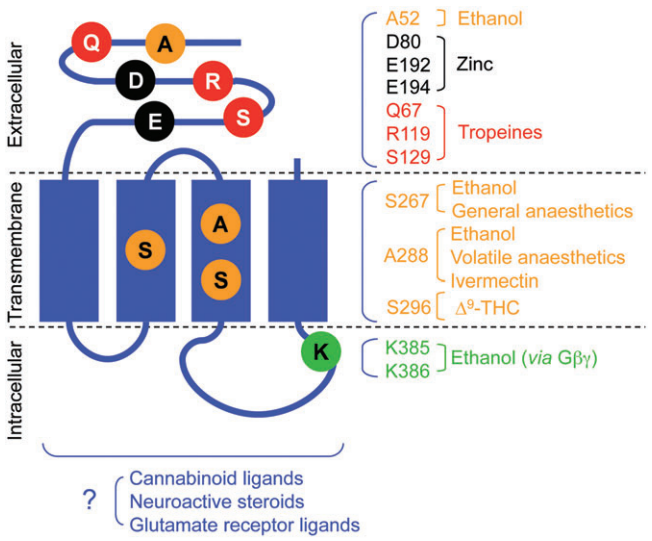

Figure 2

Molecular sites for positive allosteric modulators of GlyR function. Critical residues are shown for several allosteric modulators that exert positive effects on GlyR function. The amino acid positions described were identified in functional experiments performed on the α1-GlyR mutants. The molecular sites involved in the effects elicited by neuroactive steroids, some cannabinoid ligands (i.e. endocannabinoids and synthetic cannabinoids) and glutamate receptor ligands remain to be defined. Data are from Mascia *et al.* (1996b), Mihic *et al.* (1997), Laube *et al.* (2000), Lynch *et al.* (1998), Maksay *et al.* (2009), Lynagh and Lynch (2010), Xiong *et al.* (2011) and Yevenes *et al.* (2008).

1994). These mice do not only exhibit increased muscle tone but also show a strong hyperekplexic phenotype, very much reminiscent of human startle disease (Koch *et al.*, 1996). In fact, mutations in GlyR subunit genes are frequently found in human patients suffering from hyperekplexia/startle disease (Rees *et al.*, 2001).

There is also evidence that part of the spinal component of inflammatory hyperalgesia (i.e. an increased sensitivity to painful stimuli as a consequence of peripheral inflammation) comes from diminished glycinergic inhibition caused by the phosphorylation and inhibition of α3-GlyRs (Ahmadi *et al.*, 2002; Harvey *et al.*, 2004; Reinold *et al.*, 2005). In the spinal cord, these GlyRs are largely confined to the superficial dorsal horn, the main termination area of nociceptive afferent nerve fibres. This result fits nicely to early reports showing that exaggerated nociceptive responses can be triggered by intrathecal injection of strychnine in rats (Beyer *et al.*, 1985; Yaksh, 1989). Very recent evidence indicates that α3-GlyRs also serve an important function in brainstem respiratory control where their dephosphorylation through serotonin 5-HT1A receptor activation antagonizes opioid-induced respiratory depression (Manzke *et al.*, 2010).

Although glycinergic innervation is largely confined to the spinal cord, brainstem and cerebellum, GlyRs are widely expressed also in the forebrain, where they might become activated by ambient glycine. The affinity to glycine of un-edited receptors is normally too low for such an activation. However, high affinity receptors can be generated through cytidine deamination of GlyR transcripts (RNA editing) (Meier *et al.*, 2005). This RNA editing gives rise to novel isoforms of α2 and α3-GlyRs carrying a proline to leucine point mutation (α2[P192L] and α3[P185L]) (Meier *et al.*, 2005; Legendre *et al.*, 2009). Recent evidence suggests that such high affinity extrasynaptic GlyRs contribute to pathological changes in temporal lobe epilepsy through the silencing of hippocampal neurons (Eichler *et al.*, 2008).

In the disease states discussed above, GlyR function is affected through inherited mutations, RNA editing or post-translational modifications such as phosphorylation. Patients suffering from diseases caused by diminished inhibition would probably benefit most from facilitated glycinergic inhibition, for example, through positive allosteric GlyR modulators, while in temporal lobe epilepsy an inhibition of GlyRs might be desirable. The following sections address the mechanisms of and the molecular sites for a positive allosteric modulation.

Cannabinoid ligands

A series of reports published starting in 2005 focused on a possible role of endocannabinoids and structurally or functionally related molecules as GlyR modulators. Endocannabinoids are endogenous activators of G-protein coupled cannabinoid receptors (CB1 and CB2 receptors) (Piomelli, 2003). N-arachidonoyl ethanol amide (AEA, also known as anandamide) was the first endocannabinoid discovered, followed by 2-arachidonyl-glycerol (2-AG). Both are lipid signalling molecules, structurally related to arachidonic acid. Although many lines of evidence indicate that G-protein coupled cannabinoid receptors are the primary targets of 2-AG and AEA, several studies showed that they interact with additional targets including several ion channels (Oz, 2006).

A direct modulation of GlyR by 2-AG and AEA was first reported in hippocampal neurons where both endocannabinoids reduced the amplitude of glycinergic membrane currents and altered their rise time, desensitization and deactivation kinetics in a concentration-dependent manner (Lozovaya *et al.*, 2005). This modulation was insensitive to CB1 receptor antagonists (SR141716A) and remained intact when the recorded cell was perfused with the ubiquitous G-protein inhibitor GDP-β-S (Lozovaya *et al.*, 2005). Direct modulation of GlyR by AEA has also been found in oocytes expressing recombinant α1-GlyRs (Hejazi *et al.*, 2006). Neither SR141716A nor the cannabinoid reuptake inhibitor AM404 prevented the potentiating actions of AEA, again indicating that modulation occurred independent of CB1 receptors.

Following the identification of the two endocannabinoids 2-AG and AEA, additional, structurally related endogenous molecules were discovered (Huang *et al.*, 2001). Several of these, such as N-arachidonoyl glycine (NA-Gly) and N-arachidonoyl serine (NA-Ser), bind CB1 receptors only very weakly, but still modulate GlyRs or other ion channels (Guo *et al.*, 2008; Yang *et al.*, 2008; Barbara *et al.*, 2009). Other molecules with agonistic activity at CB1 or CB2 receptors but structurally unrelated to endocannabinoids also modulate GlyRs. Among these are some ingredients of the *Cannabis sativa* plant (Δ^9-tetrahydrocannabinol [Δ^9-THC], cannabidiol)

and several synthetic CB_1 and/or CB_2 receptor ligands (HU-210, WIN 55,212-2), which either potentiate or inhibit GlyR currents, sometimes in a subunit-specific manner (compare Table 1). Although a consistent picture has yet to emerge, these data suggest that different molecular determinants exist in the target protein for CB receptor activation and GlyR modulation.

The studies discussed above consistently found that most of the cannabinoid related compounds did not directly activate GlyRs but in most cases caused a leftward shift of the glycine concentration response curve. Another important aspect is that native $GABA_A$ receptors (in rat ventral tegmental area neurons) and recombinant $\alpha2\beta3\gamma2$ $GABA_A$ receptors expressed in *Xenopus laevis* oocytes were not modulated by AEA (Hejazi *et al.*, 2006).

First analyses of possible molecular sites for these allosteric effects were performed by Hejazi and coworkers, who found that the sensitivity to AEA was similar in homomeric α1 and heteromeric α1β-GlyRs indicating that α subunits were sufficient for this modulation (Hejazi *et al.*, 2006). They next investigated the influence of a serine to glutamine amino acid exchange in α1 at position 267 (S267Q), which was previously shown to abolish the potentiation of GlyRs by ethanol and general anaesthetics (Mihic *et al.*, 1997). No change in the potentiating action of AEA or Δ^9-THC was found in this mutant. However, mutation of the serine 267 into an isoleucine (I), which also abolishes GlyR potentiation by ethanol (Mihic *et al.*, 1997), prevented potentiation by three molecules structurally-related to Δ^9-THC (cannabidiol, HU210 and ajulemic acid) (Foadi *et al.*, 2010) suggesting a role of TM2 residues for the actions of these cannabinoids ligands. More recently, Xiong and coworkers demonstrated that potentiation of α1- and α3-GlyRs by Δ^9-THC involves a TM3 serine residue (S296 on α1 or S307 on α3-GlyRs), which likely contributes to a direct interaction of Δ^9-THC *via* hydrogen bonds (Xiong *et al.*, 2011, see also Figure 2).

Over the last several years, convincing evidence has accumulated for a direct modulatory action of cannabinoid-related compounds on recombinant GlyRs. Data supporting a significant contribution of these effects to the *in vivo* actions of (endo-)cannabinoids were however lacking until recently. The report by Xiong *et al.* (2011) provides the first evidence in support of an *in vivo* relevance showing that mice lacking α3-GlyRs exhibit a pronounced reduction in Δ^9-THC-induced analgesia. An important piece of information which is still missing in the puzzle is data demonstrating a direct amplification or prolongation of glycinergic synaptic currents by (endo-)cannabinoids.

A second issue which is particularly relevant, when cannabinoid-related molecules are considered as lead structures for the development of GlyR modulators, is their lack of specificity. Almost all of these molecules also interfere with the function of other ion channels (Oz, 2006, see also Table 2) and many of them also exhibit activity at CB1 or CB2 receptors. Again, the report by Xiong *et al.* (2011) provides new insights. Introduction of slight chemical modifications to the Δ^9-THC molecule significantly decreased affinity to CB1 receptors while fully retaining activity at GlyRs. Although comprehensive analyses of the molecular determinants are definitely still needed, the recent studies indicate that some of the cannabinoid-related molecules discussed above may constitute interesting lead compounds for the development of GlyR modulators.

Ethanol

Evidence from biochemical and electrophysiological experiments consistently indicates that GlyR currents are potentiated by ethanol at concentrations reached in humans after moderate ethanol intake. This potentiation originates from a decrease in the glycine EC_{50} without a change in maximal currents (Aguayo *et al.*, 1996; Mihic, 1999). Potentiation of GlyRs by ethanol is apparently not restricted to certain CNS areas but occurs in neurons throughout many parts of the CNS, including spinal cord, hippocampus, hypoglossal nucleus and ventral tegmental area (Aguayo *et al.*, 1996; Jiang and Ye, 2003; Eggers and Berger, 2004). Experiments performed in motoneurons from brainstem and spinal cord slices have shown that ethanol increases the amplitude of glycinergic postsynaptic currents suggesting that modulation of synaptic GlyRs by ethanol could potentially explain some of the alterations caused by ethanol in motor control and respiratory rhythms (Gibson and Berger, 2000; Ziskind-Conhaim *et al.*, 2003; Eggers and Berger, 2004).

Homomeric α1-GlyRs are more sensitive to ethanol than α2-GlyRs especially at concentrations below 100 mM (Mascia *et al.*, 1996b; Perkins *et al.*, 2008; Yevenes *et al.*, 2010). This differential sensitivity correlates well with data from neuronal preparations, in which neonatal GlyRs (mostly α2-GlyRs) were less sensitive to ethanol than mature (α1) GlyRs (Tapia and Aguayo, 1998; Eggers *et al.*, 2000; Sebe *et al.*, 2003).

Most of the knowledge about the molecular mechanisms underlying the modulation of GlyRs by ethanol originally came from the electrophysiological analysis of a set of chimeric GlyR α1/$GABA_A$ρ1 receptors (Mihic *et al.*, 1997). This seminal report identified residues in TM2 (S267) and TM3 domains (A288) which abolish the ethanol sensitivity of α1-GlyRs. Subsequent experiments combining site-specific mutagenesis, molecular modelling and covalent binding of alcohol analogues to cysteine mutants consistently determined that TM2 and TM3 residues jointly shape a water-filled cavity serving as an ethanol-binding pocket (Ye *et al.*, 1998; Mascia *et al.*, 2000). Other studies showed that the extracellular loop 2 and TM1 residues also play a role in the alcohol modulation of GlyRs, although it is not clear if they shape additional binding pockets (Crawford *et al.*, 2008; Lobo *et al.*, 2008). These studies clearly demonstrate that both ethanol binding pockets and regulatory elements for the ethanol actions are within the TM domains of GlyRs. However, ethanol sensitivity can also be effectively controlled by intracellular signalling, possibly suggesting that part of the ethanol actions occur indirectly through other ethanol-sensitive proteins. For instance, the ethanol-induced potentiation of recombinant and native GlyRs is attenuated by protein kinase C inhibitors (Mascia *et al.*, 1998; Jiang and Ye, 2003) and by ct-GRK2, a specific G-protein βγ sequester peptide (Yevenes *et al.*, 2008). The importance of the Gβγ signalling for the alcohol effects on GlyRs also has been demonstrated recently using Gβγ-insensitive α1-GlyRs, in which the mutation of two intracellular residues (KK385-386)

Table 1

Cannabinoid ligand effects on native and recombinant GlyRs

Compound	Native GlyRs(EC_{50} or concentration range examined)				Comments	Reference
AEA	↓ (0.1–1 μM) Neonatal rat hippocampal pyramidal neurons[1]				100 μM glycine (≈EC_{50})	Lozovaya *et al.*, 2005
2-AG	↓ (1 μM) Neonatal rat hippocampal pyramidal neurons[1]					
WIN 55,212-2	Little effect on amplitude, but τ_{des} and τ_{on} decreased (0.1–10 μM)					
AEA	↑ (230 nM) Acutely dissociated VTA neurons				5 μM glycine	Hejazi *et al.*, 2006
Δ^9-THC	↑ (115 nM) Acutely dissociated VTA neurons					
Δ^9-THC	↑ (0.03–1 μM) Cultured spinal neurons				10 μM glycine (≈EC_2)	Xiong *et al.*, 2011
Meth-AEA	No effect on the amplitude or kinetics of glycinergic mIPSCs from spinal cord dorsal horn slices				5 μM meth-AEA	Anderson *et al.*, 2009
	Recombinant GlyRs (EC_{50} or concentration range examined)					
	α1	**α1/β**	**α2**	**α3**		
Δ^9-THC	↑ (86 nM)	↑(73 nM)	–	–	3–5 μM glycine	Hejazi *et al.*, 2006
Δ^9-THC	↑ (0.03–50 μM)[2]	–	↑ (0.03–50 μM)[2]	↑ (0.03–50 μM)[2]	≈EC_2 (subunit-dependent)	Xiong *et al.*, 2011
AEA	↑ (320 nM)	↑ (320 nM)	–	–	3–5 μM glycine	Hejazi *et al.*, 2006
AEA	↑ (38 nM)	↑ (75 nM)	No effect	No effect	≈EC_{10} (subunit-dependent)	Yang *et al.*, 2008
HU-210	↑ (270 nM)	↑	↓ (90 nM)	↓ (50 nM)		
WIN 55,212-2	No effect	No effect	↓ (0.22 μM)	↓ (86 nM)		
N-arachidonyl-glycine	Complex action[3]	Complex action[3]	↓(3.03 μM)	↓ (1.32 μM)		
Ajulemic acid	↑ (9.7 μM)	↑ (12.4 μM)	–	–	10 μM glycine	Ahrens *et al.*, 2009b
Cannabidiol	↑ (12.3 μM)[4]	↑ (18.1 μM)[4]	–	–	10 μM glycine	Ahrens *et al.*, 2009a

[1]Qualitatively similar effects were obtained in cerebellar Purkinje neurons.
[2]EC_{50} values were not reported. The sensitivity to Δ^9-THC was α1 = α3 > α2.
[3]Complex actions with initial potentiation and subsequent inhibition.
[4]Direct activation was observed at 10- to 20-fold higher concentrations.
2-AG, 2-arachidonyl-glycerol; AEA, N-arachidonoyl ethanol amide; GlyR, glycine receptor; Δ^9-THC; Δ^9-tetrahydrocannabinol.

Table 2

GlyR positive allosteric modulators and their additional targets

Group	Representative ligands	GlyR (relative potency[1])	Additional targets	References
Volatile anaesthetics	Isoflurane, enflurane	↑↑	$GABA_A$-R, voltage-gated Ca^{2+} channels, NMDA-R, 2P-domain K^+ channels	Yamakura *et al.*, 2001; Franks, 2008
Intravenous anaesthetics	Propofol	↑↑	$GABA_A$-R, L-type Ca^{2+} channels, 11β-hydroxylase	Yamakura *et al.*, 2001; Rudolph and Antkowiak, 2004; Franks, 2008
n-alcohols	Ethanol	↑	$GABA_A$-R, NMDA-R, GIRK channels	Aguayo *et al.*, 2002; Harris *et al.*, 2008
Avermectins	Ivermectin	↑↑[2]	nAch-Rs, $GABA_A$-R, $P2X_4$-R	Krusek and Zemkova, 1994; Krause *et al.*, 1998; Adelsberger *et al.*, 2000; Silberberg *et al.*, 2007; Jelinkova *et al.*, 2008
Tropeines	Tropisetron	↑↑↑/↓↓[3]	$5HT_3$-R	Thompson and Lummis, 2007
Cannabinoid ligands	Anandamide, THC	↑↑↑/↓↓	CB-R, TRPV1, nAch-Rs/$5HT_3$-R	Piomelli, 2003; Oz, 2006
Bivalent cations	Zn^{2+}	↑↑/↓↓[3]	$GABA_A$-R, NMDA-R, TrkB-R	Smart *et al.*, 2004; Mony *et al.*, 2009; Sensi *et al.*, 2009
Glutamatergic ligands	AP5, NMDA	↑↑	NMDA-R	Dingledine *et al.*, 1999

Potentiation or inhibition ranges: ↑↑↑ or ↓↓↓, nM; ↑↑ or ↓↓, μM; ↑ or ↓, mM.
[1]Mainly from functional studies using electrophysiology (see text).
[2]Direct activator.
[3]Biphasic modulation, inhibition at >10–50 μM.

attenuated ethanol effects without altering potentiation induced by general anaesthetics (Yevenes *et al.*, 2008). Additionally, a recent report also showed that the differential ethanol sensitivity of α1- and α2-GlyRs can be better explained by a selective Gβγ modulation rather than by specific TM ethanol-binding pockets, which are conserved between these isoforms (Yevenes *et al.*, 2010). It is still a matter of debate whether direct or indirect actions are more relevant, but conceivably both the direct binding of ethanol to the receptor (reviewed in Harris *et al.*, 2008) and the indirect modulation of signalling components by ethanol (reviewed in Morrow *et al.*, 2004) could be equally important and act cooperatively to elicit the final effects on GlyRs.

The physiological importance of the molecular sites for the ethanol actions *in vivo* has been investigated through genetic approaches in mice carrying the ethanol-insensitive S267Q mutation in the α1-GlyR gene. Transgenic expression of S267Q mutated GlyR in mice decreased ethanol sensitivity in behavioural assays without inducing apparent behavioural changes in the absence of alcohol (Findlay *et al.*, 2002). Although these results support the importance of this GlyR site for alcohol actions *in vivo*, they should be interpreted cautiously as a subsequent publication of the same group investigating S267Q point-mutated ('knock-in') mice has yielded different results. Mice homozygous for the S267 point mutation exhibited spontaneous seizures and died 3 weeks after birth. Heterozygous mice survived but still displayed a severe increase in the acoustic startle responses (Findlay *et al.*, 2003). *In vitro* experiments demonstrated that the S267Q mutation in α1-GlyR significantly reduced the glycine-evoked chloride uptake in spinal cord synaptoneurosomes from heterozygous knock-in mice and dramatically disrupted receptor function at the single-channel level (Findlay *et al.*, 2003).

General anaesthetics

Many studies on recombinant GlyRs consistently demonstrate that volatile anaesthetics, such as isoflurane, enflurane, halothane and sevoflurane potentiate homomeric α1-GlyR currents at anaesthetic concentrations (Downie *et al.*, 1996; Mascia *et al.*, 1996a; Krasowski and Harrison, 1999; Yamakura *et al.*, 2001). This potentiation is not specific for α1-GlyRs, as homomeric α2-GlyRs are also sensitive to isoflurane (Harrison *et al.*, 1993), while α3-GlyRs remain to be investigated. The anaesthetics studied were unable to activate GlyRs by themselves (Harris *et al.*, 1995; Downie *et al.*, 1996; Krasowski and Harrison, 1999), but rather caused a leftward shift of the glycine concentration–response curve. These effects have been reproduced in native receptors. Isoflurane potentiated the glycine-activated currents in rat medullary neurons (Downie *et al.*, 1996), and prolonged the decay kinetics and increased the frequency of mIPSCs in rat trigeminal nucleus and spinal motoneurons (Yamashita *et al.*, 2001; Cheng and Kendig, 2002). Because glycinergic inhibition is largely confined to the hindbrain and spinal cord, it is unlikely that the loss of consciousness by volatile anaesthetics is caused via an interaction with GlyRs. However, immobility is an action of volatile anaesthetics, which is much more likely related to interactions with GlyR (Sonner *et al.*, 2003; Rudolph and Antkowiak, 2004). In line with this idea, isoflurane, enflurane and sevoflurane indeed significantly reduced spontaneous action potential firing in neurons recorded in organotypic

slice cultures of the rat ventral horn (Grasshoff and Antkowiak, 2004; 2006). An interaction with GlyRs might also be relevant for sensory processing at the spinal dorsal horn level. Extracellular recordings from wide-dynamic range neurons in intact rats have shown that halothane induced depression in the responses to thermal and mechanical noxious stimuli and that this depression was partially reversed by strychnine at doses which had no *per se* effect on wide-dynamic range neuron firing (Yamauchi *et al.*, 2002). In line with these studies, *in vivo* experiments performed in rats demonstrated that spinal GlyRs are important, although not the only, mediators of the isoflurane-induced immobility (Zhang *et al.*, 2003).

Intravenous anaesthetics may also have some effect on GlyR function, but these actions are more controversial. Propofol displayed significant modulatory activity at GlyR, but the degree of this modulation was much less than that of volatile anaesthetics, in particular at clinically relevant concentrations (Pistis *et al.*, 1997; Krasowski and Harrison, 1999). Homomeric α1-GlyRs, α1/β heteromers and homomeric α2-GlyRs are similarly sensitive to propofol (Mascia *et al.*, 1996b; Pistis *et al.*, 1997), while at least α1-GlyRs appeared to be insensitive to etomidate (Mascia *et al.*, 1996a; Pistis *et al.*, 1997). Despite the lack of information on the sensitivity of other GlyR subunits or subunit combinations, available evidence suggests that GlyRs are unlikely to play a major role in the *in vivo* effects of the intravenous anaesthetics (but see Nguyen *et al.*, 2009). This is also supported by reports, which showed that propofol-induced immobility was produced exclusively via spinal $GABA_A$ receptors (Sonner *et al.*, 2003; Grasshoff and Antkowiak, 2004).

The molecular determinants of GlyR modulation by anaesthetics have been worked out hand-in-hand with those of $GABA_A$ receptors. In fact, pioneering studies performed in the late 1990s found sites for volatile anaesthetics within TM domains of GlyRs through the analysis of chimeric receptors between the enflurane-sensitive α1-GlyRs and enflurane-insensitive ρ1-$GABA_A$ receptors (Mihic *et al.*, 1997). This and other studies have consistently shown that specific residues within TM2 and TM3 domains of α1-GlyRs potentially shape an intra-subunit cavity which serves as a general anaesthetic binding pocket and which also acts as an acceptor for ethanol and other *n*-alcohols with longer carbon chains (reviewed in Krasowski and Harrison, 1999; Lobo and Harris, 2005). Unfortunately, the knowledge regarding the molecular sites for anaesthetics on GlyRs has not yet been translated into genetic mouse models. This would be necessary in order to address the role of GlyRs on the general anaesthetic actions *in vivo*.

Glutamate

A recent publication (Liu *et al.*, 2010) provides strong evidence that glutamate, the principal fast excitatory neurotransmitter in the CNS, can act as a positive allosteric GlyR modulator. This potentiation was seen in spinal neurons in culture and in slices as well as in HEK293 cells transiently expressing GlyRs. Potentiation of GlyR currents manifested in increased single channel open probability and occurred not only by glutamate but also by NMDA, AP5, kainate, quisqualate, aspartate and kynurenic acid, while CNQX and NBQX inhibited GlyR currents indicating that the pharmacology of this modulation did not match with that of any known glutamate receptor. Experiments performed in isolated membrane patches suggest a direct binding of glutamate to the GlyR channel complex. Homomeric α1-GlyR currents were doubled by glutamate, while potentiation of α1/β heteromeric channels was in the rage of about 40–60%, possibly suggesting that the putative binding site resides on the GlyR α1 subunit. Potentiation of glycinergic inhibition by glutamate may provide an extremely fast feedback mechanism for the maintenance of balanced synaptic excitation and inhibition. Although the relevant binding sites have not yet been determined and because the findings certainly require independent verification, this previously unknown modulation may point to an additional possibility for therapeutic intervention with GlyRs.

Ivermectin

Avermectins are a family of macrocyclic lactones derived from the bacterium *Streptomyces avermitilis* and commonly used as antiparasitic and insecticide agents. They act mainly through an allosteric modulation or a direct activation of glutamate-gated chloride channels (GluCls) expressed by nematodes and insects (Wolstenholme and Rogers, 2005). Ivermectin is one member of this group whose activity on invertebrate GluCls has been characterized in detail in recombinant systems (Arena *et al.*, 1992; Cully *et al.*, 1994; 1996; Kane *et al.*, 2000). Notably, ivermectin also modulates cationic and anionic ligand-gated ion channels including GlyRs in vertebrate (see Table 2). Early electrophysiological studies showed that ivermectin influences recombinant homomeric α1 and heteromeric α1β-GlyRs at submicromolar concentrations (Shan *et al.*, 2001). More recent studies revealed that different amino acid substitutions of the TM3 residue A288 differentially affected ivermectin's action on α1-GlyR currents. The mutation A288G increased the ivermectin sensitivity to the nanomolar range, whereas the A288F substitution completely abolished its agonistic actions (Lynagh and Lynch, 2010; Figure 2). Equivalent mutations in the corresponding residue in a nematode GluCl ion channel showed a similar pattern of effects suggesting that avermectins affect vertebrate GlyRs and nematode GluCl ion channels through similar molecular sites (Lynagh and Lynch, 2010). This is further supported by the observation that homologous residues act as binding sites for ethanol and general anaesthetics on $GABA_A$ and GlyRs (reviewed in Krasowski and Harrison, 1999; Yamakura *et al.*, 2001; Lobo and Harris, 2005). The direct activation of GlyRs by ivermectin makes this compound an interesting template to design GlyR ligands with agonistic activity. However, ivermectin is not specific for GlyRs (see Table 2) and furthermore inhibits GlyRs in some preparations (Dawson *et al.*, 2000). A better understanding of the mechanisms underlying the effects of ivermectin on GlyRs will be necessary to design new analogues with improved selectivity.

Neuroactive steroids

Endogenous neurosteroids are cholesterol metabolites produced locally in the CNS. They induce fast changes in neuronal excitability through a direct interaction with ion channels. Best established is the facilitating action on $GABA_A$ receptors by 5α-pregnan-3α-ol-20-one (3α,5α-tetrahydroprogesterone; 3α,5α-THPROG; allopregnanolone) and 5β-pregnan-3α-ol-20-one (3α,5β-THPROG; pregnanolone), collectively called 3α-reduced neurosteroids. These neurosteroids neither directly activate nor potentiate GlyRs (Pistis *et al.*, 1997; Lambert *et al.*, 2001; Weir *et al.*, 2004). 3α,5β-THPROG in fact causes a small but significant inhibition of spinal GlyR currents (Wu *et al.*, 1997; Fodor *et al.*, 2006) and 3β-sulphates of pregnenolone have been shown to inhibit recombinant GlyRs expressed in oocytes (Maksay *et al.*, 2001).

By contrast, synthetic neurosteroids such as minaxolone, Org20599 and alphaxalone significantly enhance homomeric α1-GlyR currents in recombinant systems (Weir *et al.*, 2004; Ahrens *et al.*, 2008). Another recent report has shown that two synthetic pregnanolone analogues potentiate homomeric α3-GlyR currents in a voltage-dependent fashion (Jin *et al.*, 2009). Although facilitation occurred in these studies with EC_{50} values approximately 10-fold higher than those required for $GABA_A$ receptors and with generally lower efficacies (Weir *et al.*, 2004), experiments on spinal dorsal horn neurons have shown that low micromolar concentrations of minaxolone prolong the decay time kinetics of glycinergic mIPSCs in lamina II neurons (Mitchell *et al.*, 2007). At 10 μM, minaxolone additionally increased the amplitude, but not the frequency of glycinergic mIPSCs. Tonic glycinergic currents found in the same dorsal horn neurons were insensitive to minaxolone (Mitchell *et al.*, 2007). The molecular sites involved in the modulation elicited by synthetic steroids on different GlyR subtypes are still not investigated in depth.

Tropeines

Tropeines were originally identified as potent 5-HT_3 receptor antagonists. Tropisetron, also known as ICS-205,930, is one of the best-known compounds of this group. It is used mainly as an anti-emetic following chemotherapy due to its ability to target 5-HT_3 receptors involved in vomiting reflexes. An increasing body of evidence has shown that tropeines also allosterically modulate GlyRs of different subunit composition. Pioneering electrophysiological recordings in cultured spinal neurons have revealed that two tropeines, MDL-72222 and tropisetron, were able to potentiate GlyR chloride currents at nanomolar concentrations (Chesnoy-Marchais, 1996). In contrast, higher micromolar concentrations caused inhibition. Subsequent studies determined that potentiation only occurred in the presence of GlyR agonists, depended on the agonist concentration, and was also present in outside-out patches (Chesnoy-Marchais, 1996; Supplisson and Chesnoy-Marchais, 2000; Yang *et al.*, 2007). The potentiation elicited by tropisetron remained unaltered in the presence of zinc, ethanol or propofol, suggesting different binding sites and mechanisms (Chesnoy-Marchais, 1999). Interestingly, tropisetron also displayed subunit-specificity. Studies in recombinant GlyRs showed that tropisetron potentiated homomeric α1 but inhibited homomeric α2-GlyRs. Furthermore the expression of β subunits significantly increased the potentiation sensitivity of α1 and switched α2-GlyR inhibition to potentiation. These results suggest that the tropeine potentiating site lies within the α-α or α-β interface (Supplisson and Chesnoy-Marchais, 2000). Other studies also found that α2-GlyR was more effectively inhibited by tropisetron than α1-GlyR, but in contrast, they did not find any potentiation even in the presence of β subunits (Maksay *et al.*, 1999). Despite these differences, the electrophysiological data correlated well with binding studies in recombinant and native membrane preparations. For example, several tropeines have been shown to inhibit ^{3}[H]strychnine binding to GlyRs with high nanomolar affinity. In addition, they increase the glycine potency to displace ^{3}[H]strychnine, suggesting direct effects on glycine binding sites (Maksay, 1998; Maksay *et al.*, 2004). In general terms, structure-activity analysis suggests that the tropeine ring itself, the tropeine nitrogen, an aromatic ring and a carbonyl group are necessary for binding and functional potentiation (Maksay, 1998; Chesnoy-Marchais *et al.*, 2000; Maksay *et al.*, 2004). The tropeine ring, on the other hand, appears to be a primary requirement for functional inhibition (Yang *et al.*, 2007; Maksay *et al.*, 2009).

Recently, several studies addressed the location of the tropeine binding sites on GlyRs. In agreement with studies performed in 5-HT_3 receptors (Yan and White, 2005; Joshi *et al.*, 2006), tropeines appear to bind to cavities within the extracellular domain located close to the ligand binding sites. Using recombinant GlyRs, Yang *et al.* (2007) showed that mutations to N102 in the α1, but not in the β subunit (N125), abolished tropisetron inhibition without affecting the potentiation. Subsequent work performed with a structurally related tropeine (3α-(3′-methoxy-benzoyloxy) nortropane, MBN) determined that other amino acid substitutions close to the agonist-binding domain of α1-GlyRs also alter the MBN inhibition or potentiation of GlyRs (see Figure 2, Maksay *et al.*, 2009). In addition, homology models and molecular docking simulations also suggest that the biphasic modulation elicited by tropeines on GlyRs is likely to involve different docking modes in adjacent binding sites within the agonist-binding region (Maksay *et al.*, 2009).

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 GlyRs. Despite the existence of some interesting differences between the chemical determinants required for tropeine binding to GlyRs and 5-HT_3 receptors, most tropeines still bind and modulate 5-HT_3 receptors with high affinity (Maksay *et al.*, 2004). In addition, the biphasic nature of tropeine-GlyR modulation and the significant overlap between the requirements for potentiation and inhibition is also an important impediment to their use as enhances of GlyR function. A better understanding of the mechanisms underlying the potentiation of GlyR subtypes by tropeines will hopefully lead to new tropeine derivatives lacking glycinergic inhibition and 5-HT_3 receptor binding.

Zinc

The interaction of GlyRs with the cation zinc is probably at present the best characterized form of allosteric modulation of GlyR. Previous research has not only consistently established the molecular sites involved, but work in point-mutated mice has also firmly established a physiological role of this modulation. Zinc modulates GlyRs in a biphasic manner. Potentiation dominates at low (<10 μM) concentrations while inhibition occurs at higher concentrations (>10 μM) (Bloomenthal *et al.*, 1994; Doi *et al.*, 1999; Laube *et al.*, 2000). This bidirectional modulation involves different molecular sites. Potentiation is due to an increase in the affinity of GlyRs to glycine, while inhibition occurs through reduced efficacy. Amino acids involved in the potentiation by zinc are D80, E192, E194 (Lynch *et al.*, 1998; Laube *et al.*, 2000), while inhibition involves H107, H109, T112 and T133 (all positions refer to α1-GlyR) (Harvey *et al.*, 1999; Laube *et al.*, 2000; Miller *et al.*, 2005). The different GlyR isoforms differ in their susceptibility to modulation by glycine. Zinc inhibits α2-GlyR and α3-GlyR to a lesser degree than α1-GlyR. This difference is apparently due to the substitution of amino acid H107 in α1-GlyR by an asparagine residue in the corresponding positions in α2- and α3-GlyR. Generation of a point-mutated mouse carrying a D80A substitution, which largely ablates the potentiating effects of zinc without changing glycine sensitivity, expression level or receptor trafficking to the synapse, revealed a physiological function of this modulatory site (and of zinc itself) in spinal cord neuronal circuits (Hirzel *et al.*, 2006). Homozygous D80A point-mutated mice exhibit a progressive hyperekplexia-like phenotype starting about at day P12, when α1-GlyRs replace embryonic α2-GlyRs. Whether these zinc modulatory sites are suitable for therapeutic targeting is however at present not known.

Conclusions

Pharmacological modulation of glycinergic inhibition could represent a novel therapeutic strategy against a variety of diseases involving altered synaptic inhibition primarily in the spinal cord and brain stem but possibly also at supraspinal sites. Several endogenous molecules including neurotransmitters and neuromodulators, and exogenous substances such as anaesthetics and alcohols have been identified that modulate GlyR function. As most pathologies linked to GlyR dysfunction involve diminished GlyR activity, positive allosteric modulation appears desirable in the majority of cases. Most currently available GlyR modulators are rather promiscuous and by no means specific for GlyRs (compare Table 2). These compounds are therefore not suitable for a therapeutic approach targeted specifically towards GlyRs. However, for several of them, direct modulation through allosteric sites is either firmly established or very likely. The existence of putative distinct sites for allosteric modulation on GlyRs, however, indicates future possibilities for a specific modulation of GlyR subtypes by novel synthetic ligands. This perhaps optimistic view is supported by the recent report which showed that an unbiased high throughput screening approach led to the identification of several highly specific GlyR modulator peptides (Tipps *et al.*, 2010). A comprehensive mapping of the molecular sites and mechanism involved will certainly facilitate the identification and development of small molecules specifically targeting GlyRs. In the absence of high-resolution structures for GlyRs (and hence also of structural data of these receptors with bound allosteric modulators or agonists), our knowledge is restricted to what can be inferred from functional studies using recombinant mutant receptors. Alternatively, advances might also come from the analysis of structurally related channels. High-resolution X-ray structures have recently been obtained from bacterial pentameric ligand-gated ion channel (reviewed recently by Baenziger and Corringer, 2011). For the bacterial proton-activated ion channel *G. violaceus* ligand-gated ion channel crystal structures have even been obtained with a general anaesthetics bound (Nury *et al.*, 2011). It is to be hoped that such data will foster future structure-function studies on GlyRs, for example, through improved homology modelling and molecular dynamic simulations. Through these and other new approaches, the discovery and development of new synthetic drugs targeting GlyRs with improved specificity and efficacy appears to be not too far fetched.

Acknowledgement

The research of the authors on GlyRs is supported by a grant from the Swiss National Science Foundation (SNF) to H. U. Z.

Conflicts of interest

The authors state no conflict of interests.

References

Adelsberger H, Lepier A, Dudel J (2000). Activation of rat recombinant $\alpha_1\beta_2\gamma_{2S}$ $GABA_A$ receptor by the insecticide ivermectin. Eur J Pharmacol 394: 163–170.

Aguayo LG, Tapia JC, Pancetti FC (1996). Potentiation of the glycine-activated Cl^- current by ethanol in cultured mouse spinal neurons. J Pharmacol Exp Ther 279: 1116–1122.

Aguayo LG, Peoples RW, Yeh HH, Yevenes GE (2002). $GABA_A$ receptors as molecular sites of ethanol action. Direct or indirect actions? Curr Top Med Chem 2: 869–885.

Ahmadi S, Lippross S, Neuhuber WL, Zeilhofer HU (2002). PGE_2 selectively blocks inhibitory glycinergic neurotransmission onto rat superficial dorsal horn neurons. Nat Neurosci 5: 34–40.

Ahrens J, Leuwer M, Demir R, Krampfl K, Foadi N, Haeseler G (2008). The anaesthetic steroid alphaxalone positively modulates alpha1-glycine receptor function. Pharmacology 82: 228–232.

Ahrens J, Demir R, Leuwer M, de la Roche J, Krampfl K, Foadi N *et al.* (2009a). The nonpsychotropic cannabinoid cannabidiol modulates and directly activates alpha-1 and alpha-1-beta glycine receptor function. Pharmacology 83: 217–222.

Ahrens J, Leuwer M, Demir R, Krampfl K, de la Roche J, Foadi N *et al.* (2009b). Positive allosteric modulatory effects of ajulemic acid at strychnine-sensitive glycine alpha1- and alpha1beta-receptors. Naunyn Schmiedebergs Arch Pharmacol 379: 371–378.

Anderson WB, Graham BA, Beveridge NJ, Tooney PA, Brichta AM, Callister RJ (2009). Different forms of glycine- and $GABA_A$-receptor mediated inhibitory synaptic transmission in mouse superficial and deep dorsal horn neurons. Mol Pain 5: 65.

Arena JP, Liu KK, Paress PS, Schaeffer JM, Cully DF (1992). Expression of a glutamate-activated chloride current in Xenopus oocytes injected with Caenorhabditis elegans RNA: evidence for modulation by avermectin. Brain Res Mol Brain Res 15: 339–348.

Baenziger JE, Corringer PJ (2011). 3D structure and allosteric modulation of the transmembrane domain of pentameric ligand-gated ion channels. Neuropharmacology 60: 116–125.

Barbara G, Alloui A, Nargeot J, Lory P, Eschalier A, Bourinet E *et al.* (2009). T-type calcium channel inhibition underlies the analgesic effects of the endogenous lipoamino acids. J Neurosci 29: 13106–13114.

Beyer C, Roberts LA, Komisaruk BR (1985). Hyperalgesia induced by altered glycinergic activity at the spinal cord. Life Sci 37: 875–882.

Bloomenthal AB, Goldwater E, Pritchett DB, Harrison NL (1994). Biphasic modulation of the strychnine-sensitive glycine receptor by Zn^2. Mol Pharmacol 46: 1156–1159.

Buckwalter MS, Cook SA, Davisson MT, White WF, Camper SA (1994). A frameshift mutation in the mouse α1 glycine receptor gene (*Glra1*) results in progressive neurological symptoms and juvenile death. Hum Mol Genet 3: 2025–2030.

Callister RJ, Graham BA (2010). Early history of glycine receptor biology. In: mammalian spinal cord circuits. Front Mol Neurosci 3: 13.

Cheng G, Kendig JJ (2002). Pre- and postsynaptic volatile anaesthetic actions on glycinergic transmission to spinal cord motor neurons. Br J Pharmacol 136: 673–684.

Chesnoy-Marchais D (1996). Potentiation of chloride responses to glycine by three 5-HT3 antagonists in rat spinal neurones. Br J Pharmacol 118: 2115–2125.

Chesnoy-Marchais D (1999). Mode of action of ICS 205,930, a novel type of potentiator of responses to glycine in rat spinal neurones. Br J Pharmacol 126: 801–809.

Chesnoy-Marchais D, Levi S, Acher F (2000). Glycinergic potentiation by some $5\text{-}HT_3$ receptor antagonists: insight into selectivity. Eur J Pharmacol 402: 205–213.

Crawford DK, Perkins DI, Trudell JR, Bertaccini EJ, Davies DL, Alkana RL (2008). Roles for loop 2 residues of α1 glycine receptors in agonist activation. J Biol Chem 283: 27698–27706.

Cully DF, Vassilatis DK, Liu KK, Paress PS, Van der Ploeg LH, Schaeffer JM *et al.* (1994). Cloning of an avermectin-sensitive glutamate-gated chloride channel from Caenorhabditis elegans. Nature 371: 707–711.

Cully DF, Paress PS, Liu KK, Schaeffer JM, Arena JP (1996). Identification of a Drosophila melanogaster glutamate-gated chloride channel sensitive to the antiparasitic agent avermectin. J Biol Chem 271: 20187–20191.

Dawson GR, Wafford KA, Smith A, Marshall GR, Bayley PJ, Schaeffer JM *et al.* (2000). Anticonvulsant and adverse effects of avermectin analogs in mice are mediated through the γ-aminobutyric $acid_A$ receptor. J Pharmacol Exp Ther 295: 1051–1060.

Dingledine R, Borges K, Bowie D, Traynelis SF (1999). The glutamate receptor ion channels. Pharmacol Rev 51: 7–61.

Doi A, Kishimoto K, Ishibashi H (1999). Modulation of glycine-induced currents by zinc and other metal cations in neurons acutely dissociated from the dorsal motor nucleus of the vagus of the rat. Brain Res 816: 424–430.

Downie DL, Hall AC, Lieb WR, Franks NP (1996). Effects of inhalational general anaesthetics on native glycine receptors in rat medullary neurones and recombinant glycine receptors in Xenopus oocytes. Br J Pharmacol 118: 493–502.

Eggers ED, Berger AJ (2004). Mechanisms for the modulation of native glycine receptor channels by ethanol. J Neurophysiol 91: 2685–2695.

Eggers ED, O'Brien JA, Berger AJ (2000). Developmental changes in the modulation of synaptic glycine receptors by ethanol. J Neurophysiol 84: 2409–2416.

Eichler SA, Kirischuk S, Juttner R, Schafermeier PK, Legendre P, Lehmann TN *et al.* (2008). Glycinergic tonic inhibition of hippocampal neurons with depolarizing GABAergic transmission elicits histopathological signs of temporal lobe epilepsy. J Cell Mol Med 12: 2848–2866.

Findlay GS, Wick MJ, Mascia MP, Wallace D, Miller GW, Harris RA *et al.* (2002). Transgenic expression of a mutant glycine receptor decreases alcohol sensitivity of mice. J Pharmacol Exp Ther 300: 526–534.

Findlay GS, Phelan R, Roberts MT, Homanics GE, Bergeson SE, Lopreato GF *et al.* (2003). Glycine receptor knock-in mice and hyperekplexia-like phenotypes: comparisons with the null mutant. J Neurosci 23: 8051–8059.

Foadi N, Leuwer M, Demir R, Dengler R, Buchholz V, de la Roche J *et al.* (2010). Lack of positive allosteric modulation of mutated α_1S267I glycine receptors by cannabinoids. Naunyn Schmiedebergs Arch Pharmacol 381: 477–482.

Fodor L, Boros A, Dezso P, Maksay G (2006). Expression of heteromeric glycine receptor-channels in rat spinal cultures and inhibition by neuroactive steroids. Neurochem Int 49: 577–583.

Franks NP (2008). General anaesthesia: from molecular targets to neuronal pathways of sleep and arousal. Nat Rev Neurosci 9: 370–386.

Gibson IC, Berger AJ (2000). Effect of ethanol upon respiratory-related hypoglossal nerve output of neonatal rat brain stem slices. J Neurophysiol 83: 333–342.

Grasshoff C, Antkowiak B (2004). Propofol and sevoflurane depress spinal neurons in vitro via different molecular targets. Anesthesiology 101: 1167–1176.

Grasshoff C, Antkowiak B (2006). Effects of isoflurane and enflurane on $GABA_A$ and glycine receptors contribute equally to depressant actions on spinal ventral horn neurones in rats. Br J Anaesth 97: 687–694.

Grudzinska J, Schemm R, Haeger S, Nicke A, Schmalzing G, Betz H *et al.* (2005). The β subunit determines the ligand binding properties of synaptic glycine receptors. Neuron 45: 727–739.

Guo J, Williams DJ, Ikeda SR (2008). N-arachidonoyl L-serine, a putative endocannabinoid, alters the activation of N-type Ca^{2+} channels in sympathetic neurons. J Neurophysiol 100: 1147–1151.

Harris RA, Mihic SJ, Dildy-Mayfield JE, Machu TK (1995). Actions of anesthetics on ligand-gated ion channels: role of receptor subunit composition. FASEB J 9: 1454–1462.

Harris RA, Trudell JR, Mihic SJ (2008). Ethanol's molecular targets. Sci Signal 1: re7.

Harrison NL, Kugler JL, Jones MV, Greenblatt EP, Pritchett DB (1993). Positive modulation of human gamma-aminobutyric acid type A and glycine receptors by the inhalation anesthetic isoflurane. Mol Pharmacol 44: 628–632.

Harvey RJ, Thomas P, James CH, Wilderspin A, Smart TG (1999). Identification of an inhibitory Zn^{2+} binding site on the human glycine receptor α1 subunit. J Physiol 520: 53–64.

Harvey RJ, Depner UB, Wässle H, Ahmadi S, Heindl C, Reinold H *et al.* (2004). GlyR α3: an essential target for spinal PGE2-mediated inflammatory pain sensitization. Science 304: 884–887.

Hejazi N, Zhou C, Oz M, Sun H, Ye JH, Zhang L (2006). Δ9-tetrahydrocannabinol and endogenous cannabinoid anandamide directly potentiate the function of glycine receptors. Mol Pharmacol 69: 991–997.

Hirzel K, Müller U, Latal AT, Hulsmann S, Grudzinska J, Seeliger MW *et al.* (2006). Hyperekplexia phenotype of glycine receptor α1 subunit mutant mice identifies Zn^{2+} as an essential endogenous modulator of glycinergic neurotransmission. Neuron 52: 679–690.

Huang SM, Bisogno T, Petros TJ, Chang SY, Zavitsanos PA, Zipkin RE *et al.* (2001). Identification of a new class of molecules, the arachidonyl amino acids, and characterization of one member that inhibits pain. J Biol Chem 276: 42639–42644.

Jelinkova I, Vavra V, Jindrichova M, Obsil T, Zemkova HW, Zemkova H *et al.* (2008). Identification of $P2X_4$ receptor transmembrane residues contributing to channel gating and interaction with ivermectin. Pflugers Arch 456: 939–950.

Jiang ZL, Ye JH (2003). Protein kinase C epsilon is involved in ethanol potentiation of glycine-gated Cl^- current in rat neurons of ventral tegmental area. Neuropharmacology 44: 493–502.

Jin X, Covey DF, Steinbach JH (2009). Kinetic analysis of voltage-dependent potentiation and block of the glycine α3 receptor by a neuroactive steroid analogue. J Physiol 587: 981–997.

Joshi PR, Suryanarayanan A, Hazai E, Schulte MK, Maksay G, Bikadi Z (2006). Interactions of granisetron with an agonist-free 5-HT3A receptor model. Biochemistry 45: 1099–1105.

Kane NS, Hirschberg B, Qian S, Hunt D, Thomas B, Brochu R *et al.* (2000). Drug-resistant Drosophila indicate glutamate-gated chloride channels are targets for the antiparasitics nodulisporic acid and ivermectin. Proc Natl Acad Sci U S A 97: 13949–13954.

Kim EY, Schrader N, Smolinsky B, Bedet C, Vannier C, Schwarz G *et al.* (2006). Deciphering the structural framework of glycine receptor anchoring by gephyrin. EMBO J 25: 1385–1395.

Koch M, Kling C, Becker CM (1996). Increased startle responses in mice carrying mutations of glycine receptor subunit genes. Neuroreport 7: 806–808.

Krasowski MD, Harrison NL (1999). General anaesthetic actions on ligand-gated ion channels. Cell Mol Life Sci 55: 1278–1303.

Krause RM, Buisson B, Bertrand S, Corringer PJ, Galzi JL, Changeux JP *et al.* (1998). Ivermectin: a positive allosteric effector of the α7 neuronal nicotinic acetylcholine receptor. Mol Pharmacol 53: 283–294.

Krusek J, Zemkova H (1994). Effect of ivermectin on gamma-aminobutyric acid-induced chloride currents in mouse hippocampal embryonic neurones. Eur J Pharmacol 259: 121–128.

Lambert JJ, Belelli D, Harney SC, Peters JA, Frenguelli BG (2001). Modulation of native and recombinant $GABA_A$ receptors by endogenous and synthetic neuroactive steroids. Brain Res Brain Res Rev 37: 68–80.

Laube B, Kuhse J, Betz H (2000). Kinetic and mutational analysis of Zn^{2+} modulation of recombinant human inhibitory glycine receptors. J Physiol 522 (Pt 2): 215–230.

Laube B, Maksay G, Schemm R, Betz H (2002). Modulation of glycine receptor function: a novel approach for therapeutic intervention at inhibitory synapses? Trends Pharmacol Sci 23: 519–527.

Legendre P, Forstera B, Juttner R, Meier JC (2009). Glycine receptors caught between genome and proteome – functional implications of RNA editing and splicing. Front Mol Neurosci 2: 23.

Liu J, Wu DC, Wang YT (2010). Allosteric potentiation of glycine receptor chloride currents by glutamate. Nat Neurosci 13: 1225–1232.

Lobo IA, Harris RA (2005). Sites of alcohol and volatile anesthetic action on glycine receptors. Int Rev Neurobiol 65: 53–87.

Lobo IA, Harris RA, Trudell JR (2008). Cross-linking of sites involved with alcohol action between transmembrane segments 1 and 3 of the glycine receptor following activation. J Neurochem 104: 1649–1662.

Lozovaya N, Yatsenko N, Beketov A, Tsintsadze T, Burnashev N (2005). Glycine receptors in CNS neurons as a target for nonretrograde action of cannabinoids. J Neurosci 25: 7499–7506.

Lynagh T, Lynch JW (2010). A glycine residue essential for high ivermectin sensitivity in Cys-loop ion channel receptors. Int J Parasitol 40: 1477–1481.

Lynch JW (2004). Molecular structure and function of the glycine receptor chloride channel. Physiol Rev 84: 1051–1095.

Lynch JW, Jacques P, Pierce KD, Schofield PR (1998). Zinc potentiation of the glycine receptor chloride channel is mediated by allosteric pathways. J Neurochem 71: 2159–2168.

Maksay G (1998). Bidirectional allosteric modulation of strychnine-sensitive glycine receptors by tropeines and 5-HT3 serotonin receptor ligands. Neuropharmacology 37: 1633–1641.

Maksay G, Laube B, Betz H (1999). Selective blocking effects of tropisetron and atropine on recombinant glycine receptors. J Neurochem 73: 802–806.

Maksay G, Laube B, Betz H (2001). Subunit-specific modulation of glycine receptors by neurosteroids. Neuropharmacology 41: 369–376.

Maksay G, Nemes P, Biro T (2004). Synthesis of tropeines and allosteric modulation of ionotropic glycine receptors. J Med Chem 47: 6384–6391.

Maksay G, Laube B, Schemm R, Grudzinska J, Drwal M, Betz H (2009). Different binding modes of tropeines mediating inhibition and potentiation of α1 glycine receptors. J Neurochem 109: 1725–1732.

Malosio ML, Marqueze-Pouey B, Kuhse J, Betz H (1991). Widespread expression of glycine receptor subunit mRNAs in the adult and developing rat brain. EMBO J 10: 2401–2409.

Manzke T, Niebert M, Koch UR, Caley A, Vogelgesang S, Hulsmann S *et al.* (2010). Serotonin receptor 1A-modulated phosphorylation of glycine receptor α3 controls breathing in mice. J Clin Invest 120: 4118–4128.

Mascia MP, Machu TK, Harris RA (1996a). Enhancement of homomeric glycine receptor function by long-chain alcohols and anaesthetics. Br J Pharmacol 119: 1331–1336.

Mascia MP, Mihic SJ, Valenzuela CF, Schofield PR, Harris RA (1996b). A single amino acid determines differences in ethanol actions on strychnine-sensitive glycine receptors. Mol Pharmacol 50: 402–406.

Mascia MP, Wick MJ, Martinez LD, Harris RA (1998). Enhancement of glycine receptor function by ethanol: role of phosphorylation. Br J Pharmacol 125: 263–270.

Mascia MP, Gong DH, Eger EI 2nd, Harris RA (2000). The anesthetic potency of propanol and butanol versus propanethiol and butanethiol in α1 wild type and α1(S267Q) glycine receptors. Anesth Analg 91: 1289–1293.

Meier JC, Henneberger C, Melnick I, Racca C, Harvey RJ, Heinemann U *et al.* (2005). RNA editing produces glycine receptor α3(P185L), resulting in high agonist potency. Nat Neurosci 8: 736–744.

Mihic SJ (1999). Acute effects of ethanol on $GABA_A$ and glycine receptor function. Neurochem Int 35: 115–123.

Mihic SJ, Ye Q, Wick MJ, Koltchine VV, Krasowski MD, Finn SE *et al.* (1997). Sites of alcohol and volatile anaesthetic action on $GABA_A$ and glycine receptors. Nature 389: 385–389.

Miller PS, Beato M, Harvey RJ, Smart TG (2005). Molecular determinants of glycine receptor alphabeta subunit sensitivities to Zn^{2+}-mediated inhibition. J Physiol 566: 657–670.

Mitchell EA, Gentet LJ, Dempster J, Belelli D (2007). $GABA_A$ and glycine receptor-mediated transmission in rat lamina II neurones: relevance to the analgesic actions of neuroactive steroids. J Physiol 583: 1021–1040.

Mony L, Kew JN, Gunthorpe MJ, Paoletti P (2009). Allosteric modulators of NR2B-containing NMDA receptors: molecular mechanisms and therapeutic potential. Br J Pharmacol 157: 1301–1317.

Morrow AL, Ferrani-Kile K, Davis MI, Shumilla JA, Kumar S, Maldve R *et al.* (2004). Ethanol effects on cell signaling mechanisms. Alcohol Clin Exp Res 28: 217–227.

Mülhardt C, Fischer M, Gass P, Simon-Chazottes D, Guenet JL, Kuhse J *et al.* (1994). The spastic mouse: aberrant splicing of glycine receptor beta subunit mRNA caused by intronic insertion of L1 element. Neuron 13: 1003–1015.

Nguyen HT, Li KY, daGraca RL, Delphin E, Xiong M, Ye JH (2009). Behavior and cellular evidence for propofol-induced hypnosis involving brain glycine receptors. Anesthesiology 110: 326–332.

Nury H, van Renterghem C, Weng Y, Tran A, Baaden M, Dufresne V *et al.* (2011). X-ray structures of general anaesthetics bound to a pentameric ligand-gated ion channel. Nature 469: 428–431.

Oz M (2006). Receptor-independent actions of cannabinoids on cell membranes: focus on endocannabinoids. Pharmacol Ther 111: 114–144.

Perkins DI, Trudell JR, Crawford DK, Alkana RL, Davies DL (2008). Targets for ethanol action and antagonism in loop 2 of the extracellular domain of glycine receptors. J Neurochem 106: 1337–1349.

Pfeiffer F, Graham D, Betz H (1982). Purification by affinity chromatography of the glycine receptor of rat spinal cord. J Biol Chem 257: 9389–9393.

Piomelli D (2003). The molecular logic of endocannabinoid signalling. Nat Rev Neurosci 4: 873–884.

Pistis M, Belelli D, Peters JA, Lambert JJ (1997). The interaction of general anaesthetics with recombinant $GABA_A$ and glycine receptors expressed in Xenopus laevis oocytes: a comparative study. Br J Pharmacol 122: 1707–1719.

Rees MI, Lewis TM, Vafa B, Ferrie C, Corry P, Muntoni F *et al.* (2001). Compound heterozygosity and nonsense mutations in the α_1-subunit of the inhibitory glycine receptor in hyperekplexia. Hum Genet 109: 267–270.

Reinold H, Ahmadi S, Depner UB, Layh B, Heindl C, Hamza M *et al.* (2005). Spinal inflammatory hyperalgesia is mediated by prostaglandin E receptors of the EP2 subtype. J Clin Invest 115: 673–679.

Rudolph U, Antkowiak B (2004). Molecular and neuronal substrates for general anaesthetics. Nat Rev Neurosci 5: 709–720.

Ryan SG, Buckwalter MS, Lynch JW, Handford CA, Segura L, Shiang R *et al.* (1994). A missense mutation in the gene encoding the α1 subunit of the inhibitory glycine receptor in the spasmodic mouse. Nat Genet 7: 131–135.

Schmitt B, Knaus P, Becker CM, Betz H (1987). The Mr 93,000 polypeptide of the postsynaptic glycine receptor complex is a peripheral membrane protein. Biochemistry 26: 805–811.

Sebe JY, Eggers ED, Berger AJ (2003). Differential effects of ethanol on $GABA_A$ and glycine receptor-mediated synaptic currents in brain stem motoneurons. J Neurophysiol 90: 870–875.

Sensi SL, Paoletti P, Bush AI, Sekler I, Mony L, Kew JN *et al.* (2009). Zinc in the physiology and pathology of the CNS. Nat Rev Neurosci 10: 780–791.

Shan Q, Haddrill JL, Lynch JW (2001). Ivermectin, an unconventional agonist of the glycine receptor chloride channel. J Biol Chem 276: 12556–12564.

Silberberg SD, Li M, Swartz KJ (2007). Ivermectin Interaction with transmembrane helices reveals widespread rearrangements during opening of P2X receptor channels. Neuron 54: 263–274.

Simon J, Wakimoto H, Fujita N, Lalande M, Barnard EA (2004). Analysis of the set of $GABA_A$ receptor genes in the human genome. J Biol Chem 279: 41422–41435.

Sine SM, Engel AG (2006). Recent advances in Cys-loop receptor structure and function. Nature 440: 448–455.

Smart TG, Hosie AM, Miller PS (2004). Zn^{2+} ions: modulators of excitatory and inhibitory synaptic activity. Neuroscientist 10: 432–442.

Sonner JM, Zhang Y, Stabernack C, Abaigar W, Xing Y, Laster MJ (2003). $GABA_A$ receptor blockade antagonizes the immobilizing action of propofol but not ketamine or isoflurane in a dose-related manner. Anesth Analg 96: 706–712.

Supplisson S, Chesnoy-Marchais D (2000). Glycine receptor beta subunits play a critical role in potentiation of glycine responses by ICS-205,930. Mol Pharmacol 58: 763–770.

Tapia JC, Aguayo LG (1998). Changes in the properties of developing glycine receptors in cultured mouse spinal neurons. Synapse 28: 185–194.

Thompson AJ, Lummis SC (2007). The 5-HT3 receptor as a therapeutic target. Expert Opin Ther Targets 11: 527–540.

Tipps ME, Lawshe JE, Ellington AD, Mihic SJ (2010). Identification of novel specific allosteric modulators of the glycine receptor using phage display. J Biol Chem 285: 22840–22845.

Todd AJ, Spike RC, Chong D, Neilson M (1995). The relationship between glycine and gephyrin in synapses of the rat spinal cord. Eur J Neurosci 7: 1–11.

Waldvogel HJ, Baer K, Eady E, Allen KL, Gilbert RT, Möhler H *et al.* (2010). Differential localization of γ-aminobutyric acid type A and glycine receptor subunits and gephyrin in the human pons, medulla oblongata and uppermost cervical segment of the spinal cord: an immunohistochemical study. J Comp Neurol 518: 305–328.

Weir CJ, Ling AT, Belelli D, Wildsmith JA, Peters JA, Lambert JJ (2004). The interaction of anaesthetic steroids with recombinant glycine and $GABA_A$ receptors. Br J Anaesth 92: 704–711.

Wolstenholme AJ, Rogers AT (2005). Glutamate-gated chloride channels and the mode of action of the avermectin/milbemycin anthelmintics. Parasitology 131 (Suppl.): S85–S95.

Wu FS, Chen SC, Tsai JJ (1997). Competitive inhibition of the glycine-induced current by pregnenolone sulfate in cultured chick spinal cord neurons. Brain Res 750: 318–320.

Xiong W, Cheng K, Cui T, Godlewski G, Rice KC, Xu Y *et al.* (2011). Cannabinoid potentiation of glycine receptors contributes to cannabis-induced analgesia. Nat Chem Biol 7: 296–303.

Yaksh TL (1989). Behavioral and autonomic correlates of the tactile evoked allodynia produced by spinal glycine inhibition: effects of modulatory receptor systems and excitatory amino acid antagonists. Pain 37: 111–123.

Yamakura T, Bertaccini E, Trudell JR, Harris RA (2001). Anesthetics and ion channels: molecular models and sites of action. Annu Rev Pharmacol Toxicol 41: 23–51.

Yamashita M, Ueno T, Akaike N, Ikemoto Y (2001). Modulation of miniature inhibitory postsynaptic currents by isoflurane in rat dissociated neurons with glycinergic synaptic boutons. Eur J Pharmacol 431: 269–276.

Yamauchi M, Sekiyama H, Shimada SG, Collins JG (2002). Halothane suppression of spinal sensory neuronal responses to noxious peripheral stimuli is mediated, in part, by both $GABA_A$ and glycine receptor systems. Anesthesiology 97: 412–417.

Yan D, White MM (2005). Spatial orientation of the antagonist granisetron in the ligand-binding site of the 5-HT3 receptor. Mol Pharmacol 68: 365–371.

Yang Z, Ney A, Cromer BA, Ng HL, Parker MW, Lynch JW (2007). Tropisetron modulation of the glycine receptor: femtomolar potentiation and a molecular determinant of inhibition. J Neurochem 100: 758–769.

Yang Z, Aubrey KR, Alroy I, Harvey RJ, Vandenberg RJ, Lynch JW (2008). Subunit-specific modulation of glycine receptors by cannabinoids and N-arachidonyl-glycine. Biochem Pharmacol 76: 1014–1023.

Ye Q, Koltchine VV, Mihic SJ, Mascia MP, Wick MJ, Finn SE *et al.* (1998). Enhancement of glycine receptor function by ethanol is inversely correlated with molecular volume at position α267. J Biol Chem 273: 3314–3319.

Yevenes GE, Moraga-Cid G, Peoples RW, Schmalzing G, Aguayo LG (2008). A selective Gβγ-linked intracellular mechanism for modulation of a ligand-gated ion channel by ethanol. Proc Natl Acad Sci U S A 105: 20523–20528.

Yevenes GE, Moraga-Cid G, Avila A, Guzman L, Figueroa M, Peoples RW *et al.* (2010). Molecular requirements for ethanol differential allosteric modulation of glycine receptors based on selective Gβγ modulation. J Biol Chem 285: 30203–30213.

Zhang Y, Laster MJ, Hara K, Harris RA, Eger EI 2nd, Stabernack CR *et al.* (2003). Glycine receptors mediate part of the immobility produced by inhaled anesthetics. Anesth Analg 96: 97–101.

Ziskind-Conhaim L, Gao BX, Hinckley C (2003). Ethanol dual modulatory actions on spontaneous postsynaptic currents in spinal motoneurons. J Neurophysiol 89: 806–813.