Chronic Pain States: Pharmacological Strategies to Restore Diminished Inhibitory Spinal Pain Control

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Abstract

Potentially noxious stimuli are sensed by specialized nerve cells named nociceptors, which convey nociceptive signals from peripheral tissues to the central nervous system. The spinal dorsal horn and the trigeminal nucleus serve as first relay stations for incoming nociceptive signals. At these sites, nociceptor terminals contact a local neuronal network consisting of excitatory and inhibitory interneurons as well as of projection neurons. Blockade of neuronal inhibition in this network causes an increased sensitivity to noxious stimuli (hyperalgesia), painful sensations occurring after activation of non-nociceptive fibers (allodynia), and spontaneous pain felt in the absence of any sensory stimulation. It thus mimics the major characteristics of chronic pain states. Diminished inhibitory pain control in the spinal dorsal horn occurs naturally, e.g., through changes in the function of inhibitory neurotransmitter receptors or through altered chloride homeostasis in the course of inflammation or nerve damage. This review summarizes our current knowledge about endogenous mechanisms leading to diminished spinal pain control and discusses possible ways that could restore proper inhibition through facilitation of fast inhibitory neurotransmission.

INTRODUCTION

GABA: γ-aminobutyric acid

$GABA_A$: ionotropic GABA receptor

The concept of inhibitory neurons serving a critical function in spinal pain control was first proposed in the gate control theory of pain (1). Experimental proof for an endogenous inhibitory tone by fast GABAergic [i.e., γ-aminobutyric acid (GABA)-mediated] and glycinergic neurotransmission comes from behavioral experiments, which tested the effects of blockade of $GABA_A$ receptors and inhibitory glycine receptors with bicuculline and strychnine. Animals injected intrathecally (i.e., into the subarachnoid space of the spinal canal) with these antagonists responded with hyperalgesia and signs of allodynia and spontaneous pain (2, 3; see also sidebar, Hyperalgesia and Allodynia). A reduction in the inhibitory synaptic transmission at the spinal cord level thus induces pain states that have the key symptoms associated with chronic pain. At the cellular level, disinhibition increased the excitability of lamina I projection neurons (4), established functional connections from non-nociceptive primary afferent nerve fibers to normally nociception-specific neurons (5–7), and induced spontaneous epilepsy-like discharge patterns in lamina I projection neurons (4). Within the past decade, several groups demonstrated that diminished synaptic inhibition occurs endogenously during inflammatory and neuropathic pain states as well as after intense nociceptive input to the spinal cord.

MECHANISMS OF DIMINISHED INHIBITION IN PAIN

Inflammatory Pain

Prostaglandins are pivotal mediators of inflammation and pain that contribute to sensitization of pain pathways both in the periphery and in the central nervous system. Prostaglandins produced in the spinal cord following peripheral inflammation are generated mainly by the inducible cyclooxygenase isoform COX-2 (**Figure 1**). Part of their central pain-sensitizing action originates from a reduction in glycinergic pain control at the level of the dorsal horn. Work in spinal cord slices demonstrates that glycinergic neurotransmission is reduced in mice with peripheral inflammation (8). An inhibitory action on glycine receptors of prostaglandin E_2 (PGE_2) has been demonstrated in the superficial spinal dorsal horn. This inhibition occurs through activation of PGE_2 receptors of the EP2 subtype and involves protein kinase A–dependent phosphorylation of glycine receptors containing the α3 subunit (9, 10). Mice lacking the EP2 subtype of PGE_2 receptors or the glycine receptor α3 subunit, two key elements of the underlying signal transduction pathway, recover significantly faster than do wild-type mice from inflammatory hyperalgesia induced by subcutaneous zymosan A or complete Freund's adjuvant injection (9, 11–13). However, both types of knockout mice show unchanged mechanical and thermal hyperalgesia after peripheral nerve injury (12, 14). These phenotypes are very similar to those reported for mice lacking neuronal protein kinase A (15). These mice also show diminished inflammatory hyperalgesia but develop normal neuropathic pain sensitization. Supporting evidence also comes from conditional

HYPERALGESIA AND ALLODYNIA

Hyperalgesia describes a state of increased sensitivity to stimuli that are sensed as painful under normal conditions, whereas allodynia refers to pain evoked by innocuous stimuli such as light touch. On a neurophysiological basis, hyperalgesia originates from a sensitization of peripheral nociceptors or from increased responses to nociceptor activation, whereas allodynia describes pain originating from the activation of non-nociceptive fibers.

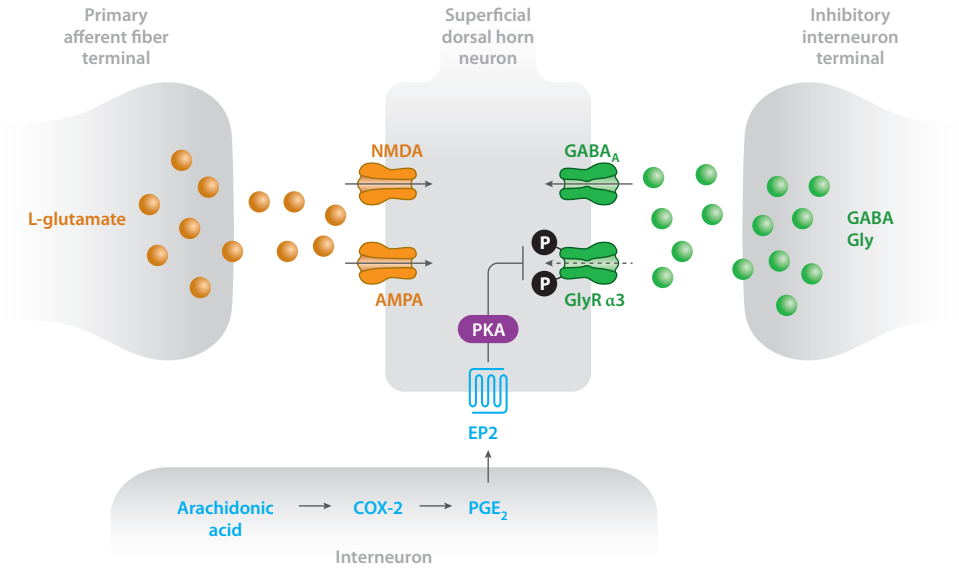

Figure 1

Possible disinhibitory mechanisms involved in inflammatory pain. Peripheral inflammation induces enzymatic production of prostaglandin E_2 (PGE_2) from arachidonic acid. PGE_2 activates prostaglandin receptors of the EP2 subtype expressed on intrinsic spinal cord neurons, which in turn activate G protein α_s and adenylyl cyclases, generating an increase in intracellular concentrations of cyclic adenosine monophosphate (cAMP). The increase in cAMP activates protein kinase A (PKA), thereby producing phosphorylation and functional inhibition of glycine receptors (GlyRs) that contain the α3 subunit. Abbreviations: AMPA, 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl)propanoic acid; COX-2, cyclooxygenase-2; GABA, γ-aminobutyric acid; Gly, glycine; NMDA, *N*-methyl-D-aspartate.

COX-2-deficient mice, which lack COX-2 specifically in the central nervous system. These mice are largely protected from inflammation-induced mechanical pain sensitization (16).

Neuropathic Pain

Diminished inhibitory neurotransmission also occurs in response to peripheral nerve damage (**Figure 2**). Activation of microglia cells in the dorsal horn and the subsequent impairment of chloride homeostasis through microglia-released brain-derived neurotrophic factor (BDNF) are critical processes in neuropathic pain sensitization. The initiating event is the recruitment and activation of microglia cells by mediators released from the central terminals of primary sensory nerve fibers. Experiments with the local anesthetic bupivacaine show that blockade of primary afferent input prevents microglia activation and subsequent hyperalgesia (17), whereby activity of non-nociceptive A fibers is apparently more important than that of C fiber nociceptors (18). Significant evidence indicates that the chemokine CCL2 [chemokine (C-C motif) ligand 2], also known as monocyte chemoattractant protein-1, and its receptor CCR2 play a critical role in this recruitment of microglia cells. CCL2 is released from the primary afferent terminals, but peripheral nerve damage also induces CCL2 expression in spinal cord neurons and astrocytes (19, 20). When injected into the spinal cord or the spinal canal, CCL2 leads to microglia activation (21), thermal hyperalgesia, and mechanical allodynia (22). Mice lacking the CCR2 receptor do not

BDNF: brain-derived neurotrophic factor

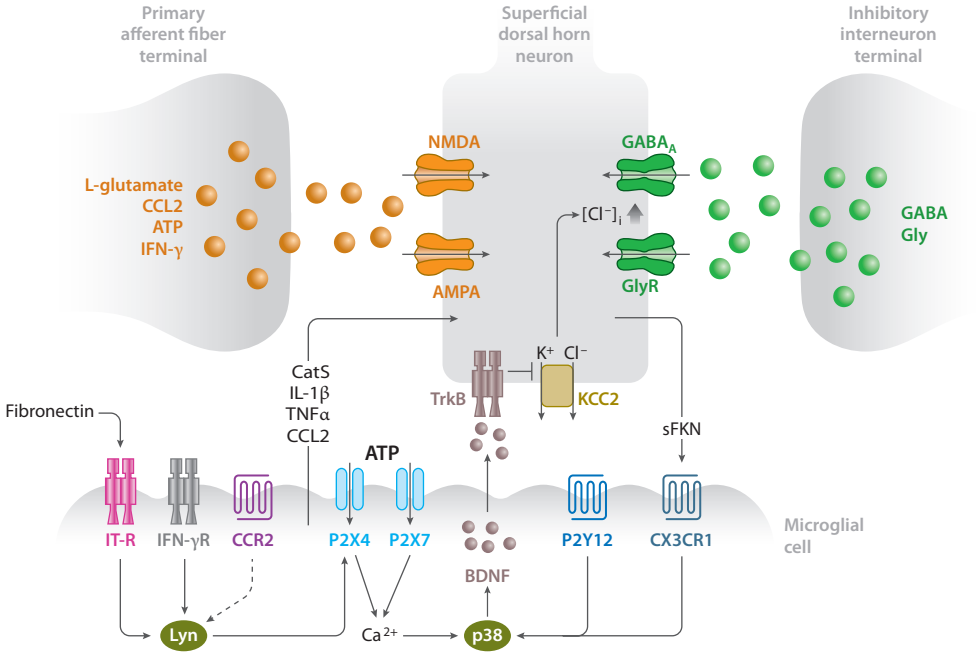

Figure 2

Mechanisms of disinhibition in neuropathic pain. Following nerve injury, activation of primary sensory nerve fibers promotes the release of the excitatory neurotransmitter L-glutamate together with other transmitters and cytokines, such as ATP, CCL2, and IFN-γ, leading to activation and proliferation of microglia through the stimulation of P2X4, P2X7, CCR2, IFN-γR, and integrin receptors. ATP-promoted activation of microglial P2X4 and P2X7 receptors stimulates the p38-MAPK signaling cascade, promoting the release of additional messengers that include BDNF, cathepsin S, TNFα, CCL2, and IL-1β. BDNF stimulates TrkB receptors expressed in superficial dorsal horn neurons to downregulate the potassium/chloride cotransporter KCC2, which ultimately leads to diminished inhibitory neurotransmission. Abbreviations: AMPA, 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl)propanoic acid; BDNF, brain-derived neurotrophic factor; CatS, cathepsin S; CCL2, chemokine (C-C motif) ligand 2; CCR2, chemokine (C-C motif) receptor 2; GABA, γ-aminobutyric acid; Gly, glycine; GlyR, glycine receptor; IFN-γ, interferon γ; IFN-γR, interferon γ receptor; IL-1β, interleukin-1β; IT-R, integrin receptor; Lyn, member of the Src family of protein tyrosine kinase; MAPK, mitogen-activated protein kinase; NMDA, *N*-methyl-D-aspartate; sFKN, soluble CX3C chemokine fractalkine; TNFα, tumor necrosis factor α.

display mechanical allodynia after nerve injury (23), whereas mice overexpressing CCL2 under the glial fibrillary protein promoter have increased nociceptive behavior (24).

Purinergic receptor–mediated signaling appears as a central process in the subsequent activation processes. Direct involvement of P2X receptors in neuropathic pain was first proposed on the basis of the finding that intrathecal injection of TNP-ATP, an antagonist of P2X receptor subtypes 1 through 4, reversed tactile allodynia in rats with injured spinal nerves (25). The role of P2X receptors is further supported by immunocytochemistry, which shows that development of pain hypersensitivity correlated well with increases in P2X4 receptor expression in dorsal horn microglia (see also Reference 26). Subsequent studies in P2X4 receptor–deficient mice and with antisense oligonucleotides directed against P2X4 receptors confirmed that these receptors were required for the development of mechanical hypersensitivity after sciatic nerve ligation (25–27). Intraspinal injection of microglia activated in vitro with ATP was sufficient to induce neuropathic

FORMALIN ASSAY

Formalin assay is a process in which a small amount of formalin is injected subcutaneously into the animal's hindpaw. This induces a nociceptive behavior consisting of repeated flexor reflexes ("flinches") and biting and licking of the injected paw. This test is often used to assess chemically induced or inflammatory pain.

pain in rodents (25). Finally, investigators demonstrated that P2X4 receptors are upregulated after nerve damage through a process that involves IFN-γ, Lyn tyrosine kinase (28, 29), the extracellular matrix protein fibronectin, and β1-integrin receptors (30).

In addition to P2X4 receptors, P2X7 receptors, which are found on resting microglia, have also been extensively studied in the context of microglia activation (31). Overexpression of P2X7 receptors in microglia can promote their activation and proliferation (32, 33), whereas pharmacological blockade or knockdown of P2X7 receptors with small interfering RNA (siRNA) impairs microglial proliferation (34). Moreover, activation of P2X7 receptors has been linked to the release of interleukin-1β (35, 36), tumor necrosis factor α (37, 38), CCL2/CCL3 (39, 40), and cathepsin S (41). In pain models, P2X7 receptor–deficient mice show normal pain sensitivity in the absence of neuropathy or inflammation (42) but do not develop thermal or mechanical allodynia following nerve ligation. P2X7 receptors therefore are probably required for initial activation of microglia, but BDNF release from microglia cells apparently depends on the upregulation and activation of P2X4 receptors. In inflammatory pain states, BDNF is released also from nociceptive fibers, but release from these fibers is apparently not relevant in neuropathic states as genetic ablation of BDNF from primary nociceptors reduces inflammatory hyperalgesia but not neuropathic pain (43). Although this study suggests that BDNF also contributes to inflammatory hyperalgesia, a link to disinhibition has not been established in inflammatory models. Instead, diminished phosphorylation of NMDA receptors and reduced activation of extracellular signal-regulated kinase were observed in mice subjected to the formalin test (43) (see sidebar, Formalin Assay, for details of this pain test).

In addition to P2X4/7 receptors, metabotropic P2Y12 receptors may also play a role in microglia activation. Following peripheral nerve injury, these receptors become upregulated in spinal microglia, and their activation promotes p38–mitogen-activated protein kinase (p38-MAPK) signaling pathways (44). Interestingly, P2Y12 receptors are expressed in resting microglia and are significantly downregulated following microglia activation (45). P2Y12 receptor–deficient mice show reduced tactile allodynia after nerve injury, without significant change in basal mechanical sensitivity (46), and microglia prepared from these mice exhibit reduced chemotaxis (45).

Another effector, fractalkine, apparently contributes to the development or maintenance of chronic pain. Peripheral nerve ligation in rats induces the expression and release of the cysteine protease cathepsin S from microglia, which releases membrane-bound fractalkine (47). Neutralizing antibodies against fractalkine can attenuate fractalkine and cathepsin S–induced pain behaviors (47). Conversely, mice deficient in the fractalkine receptor CX3CR1 are insensitive to cathepsin S–evoked or fractalkine-induced hyperalgesia and show attenuated neuropathic pain but normal responses to acute pain (48). It is therefore likely that both purinergic signaling and fractalkine/CX3CR1 act as amplifiers of microglia activation initiated by nerve injury.

An important question is, What is the nature of the ultimate messenger and event that links microglia activation to changes in neuronal excitability? Studies performed in cultured microglia have demonstrated that P2X4 receptor–evoked Ca^{2+} signals enhance BDNF synthesis and release through a MAPK-dependent pathway (49). Microglia that lack P2X4 receptors are unable

to release BDNF in response to extracellular ATP. Further studies identified the downstream mechanism that links BDNF to altered neuronal excitability. Microglia-derived BDNF downregulates the expression of the potassium/chloride cotransporter KCC2, whose activity is required to maintain the low intracellular chloride concentration that is typical of adult central neurons (50). The subsequent increase in intracellular chloride renders GABAergic synaptic currents depolarizing, as revealed by GABA-evoked Ca^{2+} signals and GABA-evoked action potential firing in rat spinal cord slices (51, 52). In vivo studies have subsequently shown that intraspinal injection of microglia cells that have been activated in vitro is alone sufficient to shift the anion reversal potential of lamina I neurons to more depolarized values and to generate allodynia, whereas microglia preincubated with siRNA against BDNF were unable to shift the reversal potential or to generate allodynia (52). Although the role of BDNF is clearly established, some intermediate contributors in this pathway, such as IFN-γ (53) and CCR2 receptors (54), also can directly impair GABAergic transmission and may contribute to central sensitization via this pathway.

KCC2: potassium/chloride cotransporter

$GABA_B$: G protein–coupled (metabotropic) GABA receptor

In addition to the mechanisms discussed above, diminished activation of metabotropic $GABA_B$ receptors may also play an important role in chronic pain states (see sidebar, Mammalian $GABA_B$ Receptors, for molecular composition). $GABA_B$ receptors are abundantly expressed in primary afferent terminals and interneurons in the superficial layers of the dorsal horn (55–58). Their activation produces analgesic effects by the inhibition of presynaptic transmitter release as well as by the inhibition of postsynaptic responses (58–62). $GABA_B$ receptor–deficient mice exhibit pronounced hyperalgesia (63, 64), which opens the possibility that downregulation of $GABA_B$ receptors might be directly involved in the diminished GABAergic inhibition associated with chronic pain states. Although no coherent picture of the regulation of $GABA_B$ receptor expression under conditions of chronic pain is available at present, there is increasing evidence that $GABA_B$ receptors may be downregulated, at least in some animal models of neuropathic pain. In a rat model of diabetic neuropathy, dorsal horn $GABA_{B1}$ mRNA and protein decrease over a time frame that coincides with the development of mechanical hyperalgesia (65). In the same model, an increased glutamatergic input from primary afferents on lamina II neurons correlates with a diminished $GABA_B$ receptor function on primary afferent terminals (66). Because the $GABA_{B1a}$ isoform of $GABA_{B1}$ is expressed predominantly at presynaptic sites (67), the downregulation of the $GABA_{B1a,2}$ receptor subtype at primary afferent terminals may contribute to an increased glutamatergic input and central sensitization. This view is supported by a selective downregulation of $GABA_{B1a}$ in the dorsal horn after spinal nerve ligation (68). Because $GABA_{B1a}$ downregulation was prevented by intrathecal injection of a p38-MAPK inhibitor, there might be a link to microglia activation in this process. However, increased glutamate receptor activity may be a direct cause for the downregulation of $GABA_B$ receptors, perhaps by switching constitutive receptor recycling to lysosomal degradation, as observed in cultured neurons (69–71).

MAMMALIAN $GABA_B$ RECEPTORS

$GABA_B$ receptors are G protein–coupled (metabotropic) receptors for GABA. They are obligatory heterodimers that consist of a $GABA_{B1}$ subunit and a $GABA_{B2}$ subunit. $GABA_{B1}$ binds the orthosteric ligand (GABA), whereas $GABA_{B2}$ interacts with allosteric modulators, binds G proteins, and is required for trafficking $GABA_B$ receptors to the plasma membrane. The $GABA_{B1}$ subunit exists in two major variants ($GABA_{B1a}$ and $GABA_{B1b}$). $GABA_{B1a,2}$ receptors are predominantly localized to presynaptic sites and modulate neurotransmitter release, whereas $GABA_{B1b,2}$ receptors primarily mediate postsynaptic inhibition.

Activity-Dependent Pain Sensitization

Apart from inflammation or neuropathy, intense nociceptive input to the spinal dorsal horn is alone sufficient to cause pain sensitization. A classical form of such activity-dependent sensitization is long-term potentiation (LTP) of excitatory synaptic transmission between C fibers and spinal projection neurons (72). This dorsal horn LTP is a likely mechanism of enhanced sensitivity to input from nociceptive fibers (i.e., to hyperalgesia), but it cannot explain painful sensations evoked by input from non-nociceptive fibers.

Diminished synaptic inhibition has also been suggested as a possible factor in activity-dependent pain sensitization. Blockade of $GABA_A$ and glycine receptors induces a hypersensitivity, in particular, to light mechanical stimuli (73). This is reminiscent of secondary hyperalgesia and allodynia seen in healthy skin areas surrounding a site of intense C fiber stimulation. This form of hyperalgesia can be evoked experimentally by intradermal injection of the TRPV1 agonist capsaicin (74, 75). We have recently suggested that intense input to the spinal dorsal horn reduces the synaptic release of glycine and GABA through the spinal production of endocannabinoids and the subsequent activation of cannabinoid (CB1) receptors located on the presynaptic terminals of dorsal horn inhibitory interneurons (76). Such a pronociceptive action of spinal endocannabinoids and CB1 receptors is also supported by work in spinal cord slices, which shows that activation of CB1 receptors facilitates substance P release in the rat spinal cord, measured as neurokinin 1 receptor internalization (77) and in line with a pronociceptive action of exogenous cannbinoid ligands in healthy human volunteers (78, 79).

STRATEGIES FOR PHARMACOLOGICAL INTERVENTION

The aforementioned studies suggest that pathological pain syndromes of different origin converge onto diminished synaptic inhibition in the dorsal horn of the spinal cord. As discussed above, diminished inhibition in the dorsal horn mimics the major symptoms of chronic pain. Thus, the pharmacological restoration of GABAergic or glycinergic inhibition at this site might be a new and rational approach to treat chronic pain states. Facilitation of glycinergic inhibition might be a particularly attractive approach because it would possibly limit the enhancement of inhibition to the spinal cord, brain stem, and a few supraspinal central nervous system sites. Unfortunately, specific glycine receptor agonists or positive allosteric modulators are not yet available (80, 81). By contrast, $GABA_A$ receptors have been extensively exploited as pharmacological targets, and ongoing developments, e.g., in the field of subtype-selective benzodiazepine site ligands, may offer new opportunities. (The term benzodiazepines in the context of this review refers not to a chemically defined group of molecules, but to agonists at the diazepam binding site of $GABA_A$ receptors.) Because many dorsal horn inhibitory neurons release both GABA and glycine from their terminals (82–84) and because most dorsal horn neurons receive both GABAergic and glycinergic inputs (85), facilitation of spinal $GABA_A$ receptors may also compensate for diminished glycinergic inhibition.

Most evidence supporting an analgesic or—more precisely—an antihyperalgesic action of spinal $GABA_A$ receptor activation comes from compounds and drugs that directly activate $GABA_A$ receptors. Local intrathecal injection of muscimol at the spinal cord level reduces nociceptive responses in rats (3, 86), and systemic administration of gaboxadol [4,5,6,7-tetrahydroisoxazolo-(5,4-c)pyridin-3-ol, also known as THIP], which activates preferentially extrasynaptic $\alpha4\beta3\delta$ receptors (87, 88), elicits strong antihyperalgesia after systemic administration in both rodents (89, 90) and humans (91, 92). For the molecular composition of $GABA_A$ receptors, see sidebar, Mammalian $GABA_A$ Receptors.

A role in nociception and pain is less clear for classical benzodiazepines, which are positive allosteric modulators of $GABA_A$ receptors. A few reports have described analgesic actions of

MAMMALIAN $GABA_A$ RECEPTORS

Mammalian $GABA_A$ receptors are pentameric ion channels assembled from a repertoire of 19 subunits designated $\alpha1$–$\alpha6$, $\beta1$–$\beta3$, $\gamma1$–$\gamma3$, δ, ε, π, θ, and $\rho1$–$\rho3$ (159). Most of these receptors contain two α subunits, two β subunits, and a single γ subunit. They are clustered in postsynaptic membranes via the scaffolding protein gephyrin (160) and mediate most of the phasic GABAergic inhibition. Benzodiazepine-sensitive $GABA_A$ receptors contain one or more of the α subunits $\alpha1$, $\alpha2$, $\alpha3$, or $\alpha5$, together with a $\gamma2$ subunit. Some $GABA_A$ receptors contain a δ subunit instead of a γ subunit. These γ subunit–lacking receptors are exclusively located at extrasynaptic sites and mediate tonic GABAergic inhibition. In the spinal dorsal horn, the most abundant $GABA_A$ receptor combinations are $\alpha2\beta3\gamma2$ and $\alpha3\beta3\gamma2$, but $\alpha1$ and $\beta2$ subunits are also expressed (161–163).

systemically administered clonazepam in chronic pain patients with musculoskeletal or cancer-related neuropathic pain (93–95). Some positive evidence also exists for analgesic actions of intrathecal midazolam in patients suffering from postoperative pain (96), labor pain (97), low back pain (98), or cancer pain (99, 100), but these reports should be considered merely as anecdotal and do not meet current controlled clinical trial standards.

What are possible explanations for the different analgesic properties of $GABA_A$ receptor agonists and benzodiazepine site agonists? One possibility is that $GABA_A$ receptors that control spinal nociception are benzodiazepine insensitive. Indeed, there is evidence that $\alpha4/\delta$-containing receptors generate a tonic GABAergic conductance in dorsal horn neurons in the spinal cord (101). Furthermore, benzodiazepine-insensitive and bicuculline-insensitive $\rho1$-containing GABA receptors have also been found to contribute to spinal control of nociception (102). However, the spinal expression levels of $\alpha4/\delta$ benzodiazepine-insensitive subunits and probably also of $\rho1$ are considerably lower than those of the benzodiazepine-sensitive subunits (103, 104). Alternatively, different antihyperalgesic efficacies of $GABA_A$ receptor agonists and benzodiazepines might originate from the absence of an endogenous GABAergic tone under resting conditions. However, this seems unlikely because the $GABA_A$ receptor antagonist bicuculline evokes strong hyperalgesia after intrathecal injection (3). In our opinion, the most likely explanation is the existence of two GABAergic pathways, one of which is tonically active and possibly saturated under resting conditions. Blockade of $GABA_A$ receptors in this pathway would cause hyperalgesia and/or allodynia, but because of the saturation, a potentiation of these receptors would not be relevant for normal sensory processing. A second pathway, possibly originating from supraspinal sites (105, 106) or dependent on excitatory input from these sites, would become active only under pathological conditions, e.g., during neuropathy or peripheral inflammation. Different lines of evidence support this idea. In mice, classical benzodiazepines exert an antihyperalgesic effect, i.e., they normalize a pathologically lowered pain threshold but do not interfere with nociceptive sensitivity in noninflamed or uninjured tissue (107). Early experiments with barbiturates, which at low concentrations also act as positive allosteric modulators of $GABA_A$ receptors, yielded results that are consistent with this idea. Although ineffective against pain when given alone, intrathecal pentothal evokes analgesia when given together with a non-analgesic dose of muscimol (108). Results from such animal studies have, however, been notoriously difficult to interpret because of confounding sedative, anxiolytic, and rewarding properties of classical benzodiazepines. In rodents, the doses required for antihyperalgesia are in fact significantly higher than those needed for anxiolysis and are typically in the same range that also produces substantial sedation (109). In humans, these "side effects" also preclude the use of classical benzodiazepines as antihyperalgesic agents.

Insights from $GABA_A$ Receptor Point-Mutated Mice

Interest in GABAergic analgesia was revived upon the availability of tools that allowed the identification of $GABA_A$ receptor isoforms responsible for spinal antihyperalgesia. This work concentrated on benzodiazepine-sensitive $GABA_A$ receptors, which contain an $\alpha1$, $\alpha2$, $\alpha3$, or $\alpha5$ subunit in addition to a $\gamma2$ subunit (see sidebar, Mammalian $GABA_A$ Receptors). Identification of the α subunits relevant for the antihyperalgesic effect of spinally applied benzodiazepines became possible through the generation of mice in which the different benzodiazepine-sensitive $GABA_A$ receptor α subunits have been rendered diazepam insensitive through the exchange of a single amino acid (110). The use of these mice demonstrated that $GABA_A$ receptors that contain the $\alpha1$ subunit ($\alpha1$-$GABA_A$ receptors) were not required for the antihyperalgesic action of intrathecal diazepam (107). This appeared particularly important because many unwanted actions of classical benzodiazepines, such as sedation, amnesia, and addiction, depend on activation of $\alpha1$-$GABA_A$ receptors (111–113) (for a review, see Reference 115). Spinal antihyperalgesic effects were most strongly attenuated in mice carrying the point mutation in the $\alpha2$ subunit; mice with point-mutated $\alpha3$and $\alpha5$ subunits also showed reduced analgesia in a neuropathic pain model but to a lesser degree (107). Although the spinal cord is likely an important site for GABAergic pain control, supraspinal sites are certainly also relevant (114). To address such supraspinal sites of action, experiments were carried out in which diazepam was administered systemically to mice that carried a point mutation in the $\alpha2$, $\alpha3$, or $\alpha5$ subunit in addition to the one in $\alpha1$-$GABA_A$ receptors (109). The presence of the point mutation in the $GABA_A$ receptor $\alpha1$ subunit in all mice avoided confounding factors related to sedation. These experiments verified a dominant contribution of $\alpha2$- and $\alpha3$-$GABA_A$ receptors to antihyperalgesia. Interestingly, analgesic actions of systemically applied diazepam were virtually identical in wild-type mice and in mice with the $\alpha1$ point mutation despite the complete absence of sedation in the point-mutated mice. This study also shows that, in mice, antihyperalgesia and sedation occur at similar doses and that both actions require doses that are significantly higher than those needed for anxiolysis (109).

Insights from Subtype-Selective Agonists

The concept of a $GABA_A$ receptor–mediated antihyperalgesia has also been addressed with subtype-selective benzodiazepine site ligands that have low or absent intrinsic activity at $\alpha1$ subunits (termed $\alpha1$-sparing benzodiazepine site ligands). Drug companies first became interested in these compounds as potential nonsedative anxiolytics. Most of these compounds are, in a strict sense, not $\alpha1$-sparing because they still bind to $\alpha1$ subunits although they lack modulating activity at these subunits. **Table 1** provides an overview of benzodiazepine site ligands with reduced activity at $\alpha1$-$GABA_A$ receptors. Following the discovery that a pharmacological enhancement of GABAergic inhibition at $\alpha2$-, $\alpha3$-, and possibly also $\alpha5$-containing $GABA_A$ receptors can revert pathological pain hypersensitivity (107), $\alpha1$-sparing benzodiazepine site ligands were evaluated not only as potential new anxiolytics but also as antihyperalgesic agents (89); for reviews, see References 115–117.

NS11394 and L-838,417 are the two subtype-selective compounds most extensively investigated for potential antihyperalgesic actions. NS11394 and L-838,417 have no (L-838,417; Reference 118) or very low (NS11394; Reference 119) intrinsic activity at the $\alpha1$-$GABA_A$ receptor. Both compounds exert substantial antihyperalgesia in various inflammatory and neuropathic pain models (89, 107). An analgesic and antinociceptive action has also been reported for SL651498 (120) in the formalin test (109) and in C fiber–evoked flexor reflexes (121). For nonselective or $\alpha1$-preferring compounds, only limited information is available. Munro et al. (116)

Table 1 Subtype-selective benzodiazepine site agonists

Compound	Absolute potentiation (%)				Relative potentiation (%)				Reference	Comments
	α1	α2	α3	α5	α1	α2	α3	α5		
Diazepam	150	280	360	120	100	100	100	100	165	αx/β1/γ2
L-838,417	1.5	42.7	42.5	38.5	1	15	12	32	118	αx/β2/γ2; GABA EC_{20}; L-838,417 (100 nM)
NS11394	11.7	73	187	94	7.8	26	52	78	119	αx/β2/γ2; GABA EC_5–EC_{25}; saturating concentration of NS11394
SL651498	105	>280	300	60	70	>100	83	50	120	α1,2,3/β2/γ2; α5/β3/γ2; 0.3 μM GABA
TPA023	1	12	33	6	0.6	4	9	5	164	αx/β3/γ2; GABA EC_{20}
HZ166[a]	167	313	346	174	48	62	45	41	123	αx/β3/γ2; GABA EC_3; HZ166 (1 μM)
Bretazenil	110	60	120	50	73	21	33	42	165	αx/β1/γ2
Zolpidem	230	210	280	15	153	75	78	13	166	αx/β1/γ2

[a]Compound 2 in Reference 123.

In the original publications, either absolute or relative efficacies (in comparison with diazepam, chlordiazepoxide, or zolpidem) were given. Corresponding missing values were calculated and should be considered as rough estimates. They may differ considerably depending on the agonist and modulator concentrations used.

tested bretazenil, a low-efficacy partial agonist with preferential activity at α1-$GABA_A$ receptors (when compared with diazepam), and zolpidem, a partial α1-preferring agonist with lack of activity at α5-$GABA_A$ receptors. Zolpidem exhibited consistent antihyperalgesic activity at a dose of 10 mg kg^{-1}, which was already sedative, whereas bretazenil failed to exhibit any antihyperalgesia. Comparing the antihyperalgesic properties and efficacy ratios of different compounds revealed that compounds with selectivity ratios (α2-$GABA_A$ receptors/α1-$GABA_A$ receptors) larger than 1 and high intrinsic activity display antihyperalgesia in the absence of sedation in rodent pain models (**Table 2**). Compounds with high selectivity and low intrinsic activity (such as L-838,417 and TPA023) show antihyperalgesic activity in some tests (122), whereas those with a selectivity ratio below 1 do not show antihyperalgesic actions at nonsedative doses. Experiments with HZ166, a recently developed benzodiazepine (123), demonstrated that a subunit specificity moderately better than that of diazepam is sufficient to elicit antihyperalgesia in the absence of significant sedation in mice (124). However, experiments with the α2/α3 subtype-selective agent MK-0343 suggest that this may be different in humans. Although anxiolytic doses of MK-0343 caused less sedation than classical benzodiazepines in rodents, this was not the case in humans, where effects of MK-0343 on saccadic peak velocity (considered a biomarker for anxiolytic efficacy; see Reference 125) were associated with significantly reduced visual alertness scores (a biomarker for sedation) (126). These results may indicate that the use of subtype-selective benzodiazepines as human analgesics require compounds with very high selectivity.

Despite the encouraging results that support a spinal antihyperalgesic activity of benzodiazepines, at least in rodents, the lack of well-documented antihyperalgesic effects of benzodiazepines in humans still challenges the concept of GABAergic analgesia in humans. There are several possible explanations for the apparent lack of analgesic effects of classical benzodiazepines in humans. The stronger sedative effects in humans might make it difficult to

Table 2 Actions of subtype-selective benzodiazepine site agonists in rodent pain models

Compound	Selectivity ratio $\alpha2/\alpha1$	Intrinsic activity at $\alpha2$	Effects in pain models	Reference(s)
Good selectivity and high intrinsic activity at $\alpha2$				
HZ166[a]	1.9	313%	Antihyperalgesic in mouse zymosan A and CCI	124
NS11394	6.2	73%	Antinociceptive in rat formalin and capsaicin test; antihyperalgesic in CFA inflammation, CCI, and SNI	89
SL651498	>2.7	>280%	Reduced electrically evoked flexor responses in rats; antinociceptive in mouse formalin test	120 109
Good selectivity and low intrinsic activity at $\alpha2$				
L-838,417	28	42.7%	Antihyperalgesic in rat zymosan A and CCI; antiallodynic in rat SNL but not TNT; antihyperalgesic but no antiallodynic effect in rat CFA	107 167 168
TPA023	12	12%	Antiallodynic in rat SNL, no antihyperalgesia in rat CFA; little effect in rat formalin, hyperalgesic in rat carrageenan and CCI	167 122
No selectivity toward $\alpha2$				
Zolpidem	0.9	210%	Antinociceptive in rat formalin and capsaicin, but only at sedative doses	89
Bretazenil	0.5	60%	No antihyperalgesia in rat CCI and SNI at nonsedative doses	89

[a]Compound 2 in Reference 123.
Abbreviations: CCI, chronic constriction injury; CFA, complete Freund's adjuvant; SNI, spared nerve injury; SNL, spinal nerve ligation; TNT, tibial nerve transsection.

unequivocally detect antihyperalgesia. However, species differences also cannot be ruled out. Differences in the antihyperalgesic properties of GABAergic compounds occur even among different strains of rats. Neuropathic hyperalgesia and allodynia are reduced efficiently by gaboxadol in Sprague-Dawley and Brown Norway rats, whereas Fischer 344 and Lewis rats are insensitive to this agent (127). It is hence possible that GABAergic control of spinal nociception is less extensive in humans than in rodents. Whether $GABA_A$ receptors are a suitable target for novel antihyperalgesic agents in humans will become clear only when highly selective compounds are available.

Negative Allosteric Modulators

Although antihyperalgesic actions of benzodiazepines have been reported consistently, at least in rodents, a rationale for a potential use of negative allosteric modulators (NAMs) may also exist. Because NAMs reduce the efficacy of GABA at $GABA_A$ receptors, analgesic effects of $GABA_A$ receptor NAMs might occur at sites or under conditions in which GABA promotes rather than alleviates pain: (*a*) In the periaqueductal gray (PAG) or the rostral ventromedial medulla (RVM), activation of $GABA_A$ receptors causes hyperalgesia, likely through an inhibition of descending antinociceptive tracts originating from the RVM and controlled by neurons in the PAG (128). (*b*) Downregulation of KCC2 induced by nerve injury shifts the chloride equilibrium potential to more positive potentials, thereby possibly causing GABA to become excitatory. Reducing activation of $GABA_A$ receptors under these conditions would reduce activation of dorsal horn neurons and possibly induce analgesia. (*c*) Dorsal horn GABAergic interneurons activate $GABA_A$ receptors on the spinal presynaptic terminals of primary nociceptors (reviewed in 129). At this site, $GABA_A$ receptors cause depolarization rather than hyperpolarization owing to a relatively high

NAM: negative allosteric modulator

Periaqueductal gray (PAG): part of the endogenous pain control system in the midbrain

Rostral ventromedial medulla (RVM): part of the endogenous pain control system in the brain stem

Table 3 Negative allosteric modulators of $GABA_A$ receptors

	Absolute inhibition (%)				Reference(s)	Comment
	α1	α2	α3	α5		
FG-7142	47	38	40	35	169	αx/β3/γ2; EC_{20}
α5IA-II	14	7	17	45	170	αx/β3/γ2; EC_{20}
DMCM	71	53	62	57	171	αx/β3/γ2; EC_{20}

Abbreviation: DMCM, methyl-6,7-dimethoxy-4-ethyl-beta-carboline-3-carboxylate.

intracellular chloride concentration maintained in these cells by the chloride importer NKCC-1. This primary afferent depolarization normally inhibits synaptic transmission but may also exaggerate pain when it becomes sufficiently strong to trigger action potentials and elicit what are termed dorsal root reflexes (130, 131).

A recent study reported antihyperalgesic or analgesic actions of FG-7142 and α5IA-II, two $GABA_A$ receptor NAMs (**Table 3**) in rat pain models (122). Chemically induced nociception was investigated in the rat formalin test, and inflammatory pain was assessed after subcutaneous injection of carrageenan (as changes in weight-bearing deficits of the inflamed paw and changes in mechanical response thresholds). Neuropathic pain was studied in rats with chronic constriction injury of the sciatic nerve and quantified as changes in weight-bearing deficits and mechanical withdrawal thresholds. FG-7142, which does not discriminate among the different benzodiazepine-sensitive subunits (132), reduced nociceptive responses in the formalin test and significantly decreased weight-bearing deficits in rats with paw inflammation. Effects in neuropathic rats were less pronounced. The α5-specific NAM α5IA-II (133) caused statistically significant pain relief only in the inflammatory pain model. A third nonselective but more effective NAM (methyl-6,7-dimethoxy-4-ethyl-beta-carboline-3-carboxylate, also known as DMCM) was investigated only in the formalin test, in which it failed to exert statistically significant effects at subconvulsive doses.

The fact that both FG-7142 and α5IA-II were more effective in the inflammatory pain model than in the neuropathic pain model suggests that the analgesic action of these compounds did not require the neuropathy-induced switch of GABA's action from hyperpolarization to depolarization. A possible role of presynaptic $GABA_A$ receptors expressed on the spinal terminals of primary nociceptors has been addressed recently through the use of mice that lack benzodiazepine-sensitive α2-$GABA_A$ receptors in primary nociceptors (*sns*-$\alpha2^{-/-}$ mice). Most $GABA_A$ receptors in primary nociceptors are of the α2 subtype (103), but mRNA encoding for α3 and α5 subunits has also been detected in murine and human dorsal root ganglia (i.e., where the somata of primary sensory and nociceptive neurons reside) (134, 135). These *sns*-$\alpha2^{-/-}$ mice showed reduced rather than enhanced antihyperalgesia in response to intrathecally injected diazepam in an inflammatory pain model (subcutaneous zymosan A injection), whereas antihyperalgesia was unchanged in nerve-injured mice. These results cast some doubts on the idea that $GABA_A$ receptors on the spinal terminals of primary nociceptors have a significant pronociceptive role in inflammatory pain states and instead suggest that facilitation of $GABA_A$ receptor activation on spinal nociceptor terminals is analgesic. In our opinion, the most attractive explanation for the antihyperalgesic effect of the NAMs of $GABA_A$ receptors is a reduction in the GABAergic inhibition of descending antinociceptive tracts. Blockade of $GABA_A$ receptors in the PAG indeed induces analgesia (136), whereas injection of midazolam reverses fear-conditioned hypoalgesia (137).

To better judge the mechanism and the analgesic potential of $GABA_A$ receptor NAMs, it will be helpful to learn more about the sites of these actions (e.g., supraspinal, spinal, or even

peripheral); to assess the contribution of different $GABA_A$ receptor isoforms; and finally, and perhaps most importantly, to determine whether these actions can be reversed by the benzodiazepine site antagonist flumazenil (Ro 15-1788), which reverses the action of NAMs at $GABA_A$ receptors (138). A detailed knowledge of the $GABA_A$ receptor isoforms that are responsible for such effects would also be required to avoid the proconvulsive and anxiogenic effects of NAMs (139, 140). α5IA-II is devoid of anxiogenic properties, and its analgesic efficacy suggests that α5-$GABA_A$ receptors might be particularly relevant for potential analgesic effects of $GABA_A$ receptor NAMs. The relevance of α5-$GABA_A$ receptors for the analgesic effects of $GABA_A$ receptor NAMs would be consistent with a major contribution of α2- and α3-$GABA_A$ receptors to antihyperalgesia by positive allosteric modulators (115).

OUTLOOK

In addition to the recent advances in the development of subtype-selective $GABA_A$ receptor modulators, other less advanced but nevertheless interesting developments are directed toward the targeting of other proteins in spinal inhibitory synapses (**Figure 3**). Positive allosteric modulation of glycine receptors and of GABA, glycine, or chloride transporters may be other potentially useful approaches.

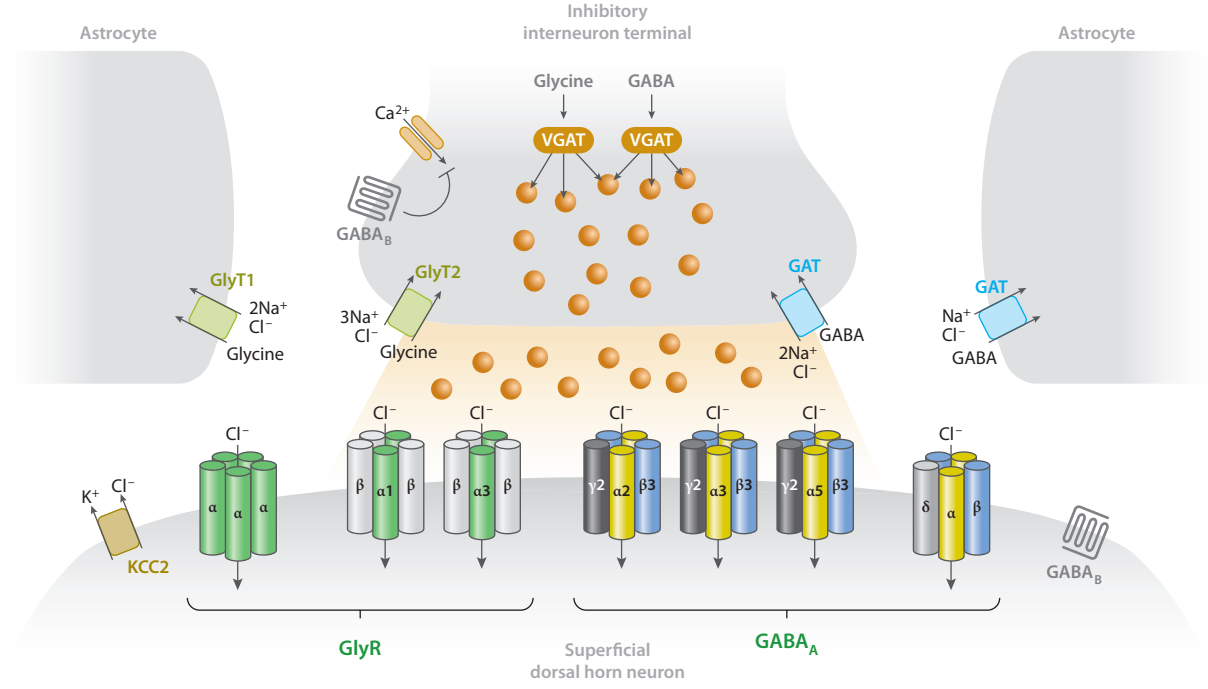

Figure 3

Potential drug targets in spinal inhibitory synapses. In addition to the different isoforms of synaptic and extrasynaptic $GABA_A$ and glycine receptors (GlyRs), $GABA_B$ receptors and glycine, GABA, and chloride transporters are potential drug targets. $GABA_B$ receptor agonists or positive modulators might be used to reduce transmitter and mediator release from nociceptor terminals. Inhibition of plasma membrane glycine and GABA transporters (GlyT1/2 and GAT1/3) could be used to strengthen synaptic inhibition, and positive allosteric modulators of KCC2 might restore the transmembrane chloride gradient required to maintain an inhibitory action of $GABA_A$ and glycine receptors. Abbreviations: GABA, γ-aminobutyric acid; GAT, plasma membrane–bound GABA transporter; GlyT, plasma membrane–bound glycine transporter; KCC2, potassium/chloride cotransporter; VGAT, vesicular GABA transporter.

GlyT1/2: plasma membrane–bound glycine transporter 1/2

GAT: plasma membrane–bound GABA transporter

Positive allosteric modulation of glycine receptors might restrict pharmacological enhancement of inhibition largely to the spinal cord or the brain stem, thereby helping to avoid unwanted effects, such as sedation. Drugs that specifically target glycine receptors are still lacking, but reports that have tested the effects of zinc, volatile anesthetics, tropeines, or cannabinoid-related molecules have identified sites for positive allosteric modulation that might be suitable for drug therapy (80, 81).

Enhancement of glycine-mediated inhibition by the use of inhibitors of glycine transport is another approach for which proof-of-concept data have been obtained. Uptake of glycine in the central nervous system is accomplished by two transporters, GlyT1 and GlyT2, whose function has been studied extensively in knockout mouse models (reviewed in 141). GlyT1-deficient mice show a phenotype consistent with increased glycinergic inhibition, whereas GlyT2-deficient mice exhibit signs of diminished glycinergic inhibition. These phenotypes correspond well to the different roles of the two glycine transporters. GlyT1 primarily mediates the removal of glycine from the extracellular space (e.g., after synaptic release) into glia and neurons, whereas GlyT2 provides glycine for uptake into presynaptic storage vesicles. Despite these different functions of GlyT1 and GlyT2, antinociceptive effects have been reported for both GlyT1 blockers (ORG25935 and sarcosine) and GlyT2 blockers (ORG25543 and ALX1393) in various pain models (142–146).

Inhibition of plasma membrane GABA transporters enhances tonic GABAergic inhibition at different brain sites—which include the hippocampus (147), cerebral cortex (148), cerebellum (149)—and enhances fast GABAergic synaptic transmission in the cortex (148). Peripheral neuropathy increases expression of the GABA transporter GAT1 in the dorsal horn of the spinal cord (150) and in the gracile nucleus of the brain stem (151), and carrageenan injection into the facial skin stimulates expression of GAT1 and GAT3 in the spinal trigeminal nucleus (152). Mice deficient in GAT1 are hypoalgesic (153), and pharmacological inhibition with NO-711 of GAT1 activity reduces excitatory transmitter release in the dorsal horn (154).

Because downregulation of KCC2 and subsequent intracellular chloride accumulation are major contributors to pathological pain, pharmacological enhancement of KCC2 activity through positive allosteric modulators (155) or through interference with endogenous regulatory pathways (156–158) might also constitute attractive approaches.

Subtype-selective benzodiazepine ligands are the most advanced of the potential therapeutic options discussed in this review. Because α1-$GABA_A$-receptor-sparing agonists are already under development as potentially nonsedative anxiolytics, it is hoped that compounds will soon become available for proof-of-principle studies in experimental human pain models or pain patients, potentially revealing a new therapeutic approach to chronic pain.

SUMMARY POINTS

1. Diminished GABAergic and/or glycinergic inhibition is a major contributor to pathological pain states of inflammatory and neuropathic origin.
2. Facilitation of spinal GABAergic synaptic inhibition reverses inflammatory and neuropathic hyperalgesia in rodent models of inflammatory and neuropathic pain.
3. Data from studies that have assessed $GABA_A$ receptor point-mutated mice indicate that α2- and α3-containing $GABA_A$ receptors mediate these spinal antihyperalgesic actions.
4. Subtype-selective (α1-sparing) benzodiazepine site agonists show significant antihyperalgesic effects in rodents in the absence of sedation.

FUTURE ISSUES

1. Which degree of subtype selectivity is required to avoid sedative effects of novel $GABA_A$ receptor modulators?
2. Is the antihyperalgesic action of subtype-selective $GABA_A$ receptor modulators that is found in rodents also present in humans?
3. Which sites are responsible for the recently described analgesic action of $GABA_A$ receptor NAMs?
4. Which $GABA_A$ receptor isoforms mediate the analgesic action of $GABA_A$ receptor NAMs?

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