Opinion

Cell PRESS

GABAergic analgesia: new insights from mutant mice and subtype-selective agonists

Hanns Ulrich Zeilhofer, Hanns Möhler and Alessandra Di Lio

Institute of Pharmacology and Toxicology, University of Zurich, CH-8057 Zurich, Switzerland
Institute of Pharmaceutical Sciences, Swiss Federal Institute of Technology (ETH Zurich), CH-8093 Zurich, Switzerland

γ-Aminobutyric acid (GABA) is the most abundant inhibitory neurotransmitter in the brain where it regulates many physiological functions including sleep, anxiety, reward and memory formation. GABAergic neurons and ionotropic $GABA_A$ receptors are also found in the spinal cord dorsal horn where they control the propagation of pain signals from the periphery to higher central nervous system areas. Recent evidence indicates that diminished inhibitory control at this site is a major factor in chronic pain syndromes. So far, this knowledge could not be translated into clinical pain therapy, probably because of the widespread actions of GABA in the central nervous system. The identification of $GABA_A$ receptor subtypes responsible for spinal antihyperalgesic effects has recently opened new avenues for the development of subtype-selective modulators of $GABA_A$ receptors. First results raise hopes that such compounds will be active against inflammatory and neuropathic pain but devoid of many of the side-effects of the established benzodiazepine-like drugs.

Introduction

γ-Aminobutyric acid (GABA; see Glossary) and glycine are the two fast inhibitory neurotransmitters in the mammalian spinal cord. Upon binding to their receptors they activate chloride-permeable ion channels to hyperpolarize neurons and to impair the dendritic propagation of excitatory signals through activation of a shunting conductance. Recent evidence suggests that diminished GABA- and glycine-mediated inhibition in the spinal dorsal horn is a hallmark of pathological pain of various origins [1]. Under healthy conditions, GABAergic and glycinergic interneurons serve as gate-keepers controlling the relay of nociceptive signals from the periphery through the spinal cord to higher central nervous system (CNS) areas and preventing the excitation of normally 'pain-specific' projection neurons by innocuous stimuli (Figure 1). Early studies in the 1980s have shown that blockade of spinal $GABA_A$ or strychnine-sensitive glycine receptors induces signs of severe pain hypersensitivity in rats [2,3], whereas mice carrying deletions in GABA and glycine receptor channel genes show increased pain sensitivity [4–6]. More recent studies from different laboratories have demonstrated that diminished inhibitory control of pain also occurs endogenously in rodent models of inflammatory and neuropathic pain. Recently proposed mechanisms include the activation of microglia in response to peripheral nerve damage, which render GABAergic and glycinergic input less inhibitory through diminished expression of the potassium chloride co-transporter KCC-2 in dorsal horn neurons and a subsequent disruption of the neuronal transmembrane chloride gradient [7,8]. Moreover, peripheral nerve damage has been reported to cause disinhibition through apoptosis of inhibitory interneurons [9,10] (see also Ref. [11]). There is also strong evidence for a loss of spinal synaptic inhibition triggered by peripheral inflammatory processes, in which spinal production of prostaglandin E2 causes a phosphorylation and subsequent blockade of glycine receptors in the superficial dorsal horn [12,13], the first site of synaptic processing in the pain pathway.

Both inflammatory pain and nerve injury-induced neuropathic pain thus seem to converge onto reduced synaptic inhibition. A rational approach to such syndromes of pathological pain could, therefore, aim at the pharmacological restoration of synaptic inhibition. In addition to inhibitory glycinergic synapses, which are found mainly in the spinal cord and brain stem but for which so far no well-suited modulators are available, ionotropic $GABA_A$ receptors constitute a promising target.

$GABA_A$ receptors as targets in pathological pain

In fact, a crucial role of inhibitory neurotransmission in spinal pain control was first proposed more than 40 years ago in the so-called 'gate control theory of pain' [14], but it has not yet been translated into pain therapy. The reasons

Glossary

Allodynia: painful sensation evoked by normally non-painful stimuli (e.g. light touch).
Anxiolytic: relieving anxiety.
Benzodiazepines: a class of positive allosteric modulators of $GABA_A$ receptors.
fMRI: functional magnetic resonance imaging.
GABA: γ-aminobutyric acid.
$GABA_A$ receptor: chloride permeable ion channel activated by GABA.
Hyperalgesia: increased sensitivity to noxious (painful) stimuli.
KCC-2: potassium chloride exporter, expressed in central neurons, keeps intracellular chloride concentrations low and allows GABA to hyperpolarize neurons.
Nociception: Physiological responses triggered by noxious (potentially tissue damaging) stimuli.

Corresponding author: Zeilhofer, H.U. (zeilhofer@pharma.uzh.ch).

0165-6147/$ – see front matter doi:10.1016/j.tips.2009.05.007 Available online xxxxxx

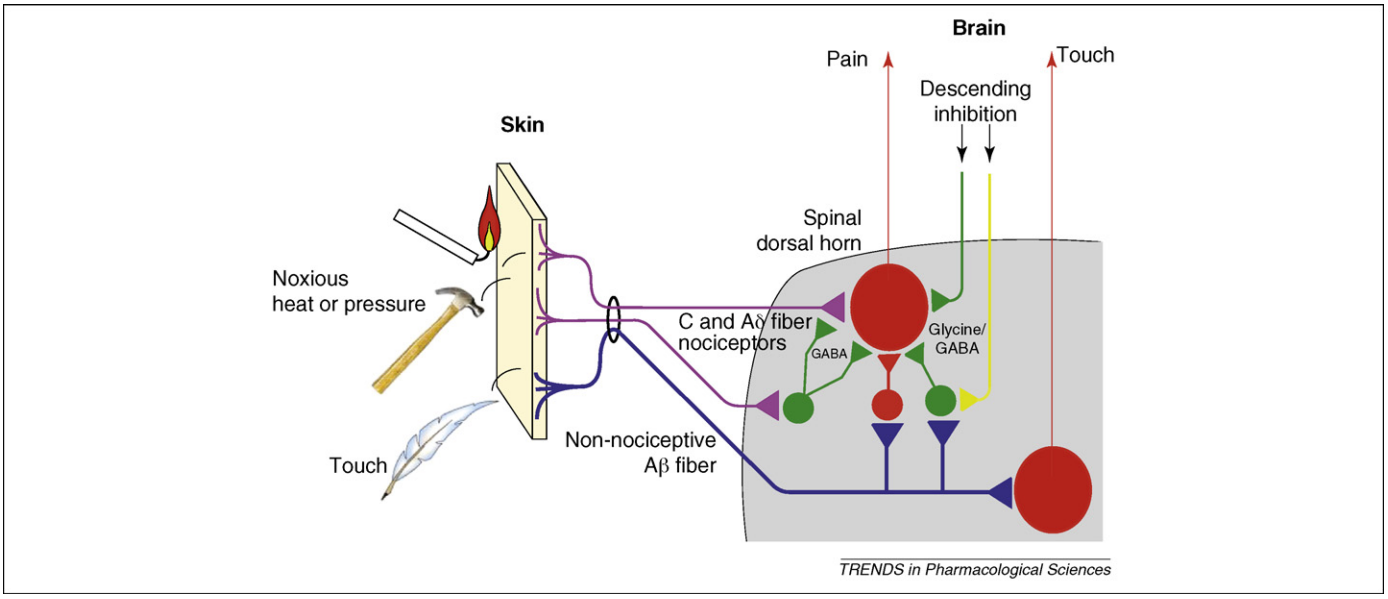

Figure 1. Pain control by spinal inhibitory neurotransmission. Noxious (potentially tissue damaging) stimuli (e.g. heat and strong mechanical pressure) are sensed by unmyelinated C or thinly myelinated Aδ nerve fibers (nociceptors, magenta), whereas light mechanical stimuli (such as touch) are sensed by thick myelinated Aβ fibers (blue). Nociceptive and non-nociceptive primary afferent nerve fibers contact different populations of spinal projection neurons (large red symbols) located in the superficial and deep dorsal horn, respectively. Aβ fibers also form polysynaptic connections with normally pain-specific projection neurons through local excitatory interneurons (small red symbols) [48,49]. Under healthy conditions, this polysynaptic input onto normally pain-specific projection neurons is counterbalanced by input from inhibitory interneurons (green). These inhibitory neurons control spinal nociception through different mechanisms. They inhibit the transmission of nociceptive signals from primary afferent nociceptors onto postsynaptic projection neurons through axo-axonic synapses with primary afferent nociceptors (presynaptic inhibition) and reduce the excitability of intrinsic dorsal horn neurons through axo-dendritc and axo-somatic synapses (postsynaptic inhibition). Presynaptic inhibition is mediated by purely GABAergic synapses, whereas both GABA and glycine contribute to postsynaptic inhibition. Inhibitory dorsal horn neurons are activated by input from primary afferent nerve fibers and through descending antinociceptive pathways (yellow). There is also good evidence for the existence of direct antinociceptive GABAergic and glycinergic pathways descending from the brain stem [50,51]. The vast majority of $GABA_A$ receptors in the superficial dorsal horn are of the α2 and/or α3 containing subtype (see also Figure 2b).

for this are probably several-fold. Plenty of evidence indicates that increasing $GABA_A$ receptor-mediated inhibition in the CNS alleviates pain both in rodents and in humans (for review, see Refs [15,16]). Several classical benzodiazepines (mainly clonazepam and midazolam) are analgesic after spinal injection in rodent models and in patients with postoperative pain. Furthermore, tiagabine, a GABA reuptake inhibitor, reduced pain-related behaviors in mice after intrathecal injection in tests of acute and tonic nociception [17] and neuropathy-associated pain in humans [18]. Vigabatrin, a GABA transaminase inhibitor that reduces GABA degradation, is antinociceptive in several rodent models [19,20]. Owing to the widespread expression of $GABA_A$ receptors in the CNS, however, unrestricted activation of all $GABA_A$ receptors in the CNS leads to strong sedation and various other side-effects. Subtype-selective agents directed towards only a subset of $GABA_A$ receptors might provide a potential solution to this dilemma (Box 1).

Early work on $GABA_A$ receptor-subtype-specific analgesia focused on receptors containing the δ subunit in association with the α4 subunit. These benzodiazepine-insensitive receptors were found to mediate the analgesic and hypnotic actions of 4,5,6,7-tetrahydroisoxazolo-[5,4-c]pyridine-3-ol (THIP) (also called gaboxadol), which acts as an agonist at the GABA site [21]. Indeed, mice lacking the α4 subunit by targeted gene disruption were insensitive to the sedative and analgesic effects of gaboxadol [22]. Although the α4δ receptors are of low abundance in brain, they are specifically expressed in thalamus, dentate gyrus and striatum. In the control of pain perception and vigilance, δ-containing $GABA_A$ receptors in thalamus are considered to provide a 'gain control' in thalamocortical pathways for regulation of the sensory information reaching the cerebral cortex [23]. Owing to the extrasynaptic location of δ subunit-containing receptors, these effects reflect tonic GABA-mediated inhibition.

Interestingly, at recombinant α4β3δ $GABA_A$ receptors, the maximal receptor response *in vitro* induced by gaboxadol was higher than that for GABA, suggesting that GABA acts as a partial agonist on these receptors [24]. Mutant mice lacking the β3 subunit gene displayed a drastic phenotype: their very low life expectancy was associated with epilepsy, cleft palate and hypersensitive behavior [4], including thermal hyperalgesia and tactile allodynia in the survivors [5]. Not unexpectedly, gaboxadol failed to induce antinociception in these mutants [5]. Gaboxadol exhibited potent analgesic effects in postoperative patients and in patients with chronic pain of malignant origin, but also showed strong sedation in most of the patients [25]. It has been developed as a sleep-promoting agent, but development was discontinued in 2007.

Pain studies in $GABA_A$ receptor mutant mouse

A more recent development started in the 1990s with the discovery that benzodiazepines, a class of positive allosteric modulators of $GABA_A$ receptors, do not act through a single $GABA_A$ receptor subtype but through four different subtypes defined by the α subunit (α1, α2, α3 or α5), which together with the γ2 subunit constitutes the benzodiazepine binding site (Box 1). This finding has potentially opened an entirely new benzodiazepine pharmacology. The

Box 1. Molecular composition of $GABA_A$ receptors

Ionotropic $GABA_A$ receptors are heteropentameric chloride-permeable ion channels composed from a repertoire of 19 subunits giving (theoretically) rise to an overwhelmingly large receptor diversity. Most $GABA_A$ receptors in the CNS are benzodiazepine-sensitive and contain two α subunits (α1, α2, α3 or α5), two β subunits and one γ2 subunit, of which the latter together with one of the α subunits forms the benzodiazepine binding site. A smaller subset of $GABA_A$ receptors that is mainly located extrasynaptically contains, instead of γ2, a δ subunit together with β3 and α4 or α6 (in the cerebellum). α4β3δ $GABA_A$ receptors are benzodiazepine insensitive, but display preferential potency and intrinsic activity for 4,5,6,7-tetrahydroisoxazolo-[5,4-c]pyridine-3-ol (THIP or gaboxadol) [24], which possesses sleep-promoting and analgesic properties [21] (Figure I).

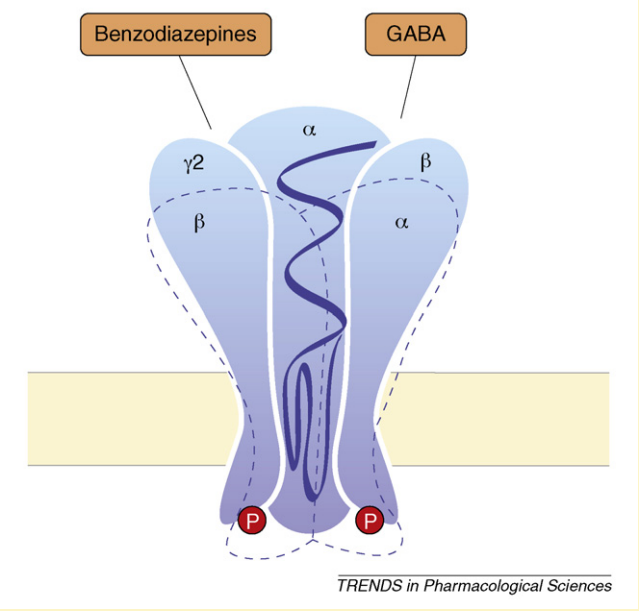

Figure I. Molecular composition of benzodiazepine-sensitive $GABA_A$ receptors.

generation of four $GABA_A$ receptor point-mutated mice carrying diazepam-insensitive $GABA_A$ receptor α subunits has greatly facilitated the attribution of the different *in vivo* actions of benzodiazepines to different $GABA_A$ receptor isoforms [26]. Importantly, these point-mutated $GABA_A$ receptors respond normally to GABA but are insensitive to the facilitating action of diazepam [27]. These mice allowed attribution of the sedative actions of benzodiazepines to $GABA_A$ receptors containing α1 subunits [28], whereas the anxiolytic actions require the activation of $GABA_A$ receptors containing α2 subunits [29]. By using these mice, it was also possible to attribute the antihyperalgesic effects of diazepam in the spinal cord to $GABA_A$ receptors containing α2 and α3 subunits [30] (Figure 2a,b). Importantly, in a variety of pain models, point mutation of the (sedative) α1 subunit does not reduce the antihyperalgesic actions of diazepam [30,31]. In addition, spinally injected diazepam does not exert general antinociceptive actions in non-inflamed or non-injured animals but only reverses pathologically increased pain sensitivity, a finding that is in good agreement with the notion that benzodiazepines are generally not analgesic [15].

The use of α1 point-mutated mice, which are resistant to the sedative properties of systemic diazepam, also allowed for the first time assessment of the potential antihyperalgesic actions of diazepam after systemic administration in the absence of confounding sedation. In wild-type mice, the analgesic and sedative effects of systemic diazepam occurred with almost the same dose dependence, yet analgesia was completely retained in the non-sedated α1 point-mutated mice [31].

The pronounced efficacy of spinally injected benzodiazepines bears also interesting neurobiological implications. In the case of the inflammatory pain model, it suggests that the positive modulation of $GABA_A$ receptors can compensate also for the reduction in glycine-mediated neurotransmission that underlies inflammatory hyperalgesia [12,13]. It is probably the simultaneous release of GABA and glycine from the same synaptic terminals [32,33] that enables $GABA_A$ receptor modulation to compensate for diminished glycine-mediated neurotransmission. In the case of neuropathic pain, we have outlined earlier that a depolarizing shift in the transmembrane chloride gradient of dorsal horn neurons is a possible mechanism of neuropathic pain [7,8]. However, a net excitatory effect of GABA released at dorsal horn synapses would be difficult to reconcile with the antihyperalgesic effect of spinally injected benzodiazepines. Their pronounced efficacy in neuropathic pain models hence suggests that the net effect of GABA in the spinal dorsal horn remains inhibitory also in neuropathic pain conditions.

Subtype-selective benzodiazepine-site ligands

Recent advances in the field of subtype-selective benzodiazepine site ligands have raised hopes that the findings obtained in $GABA_A$ receptor mutated mice might translate into new pain therapies. Several non-sedative (α1 sparing) benzodiazepines site agonists have been developed, mainly in the quest for novel non-sedative anxiolytic drugs. According to data obtained in the mutant mouse studies, such compounds should also possess substantial antihyperalgesic activity. Indeed, several of these compounds (Table 1) showed pronounced antihyperalgesia in several rodent pain models after systemic administration without sedation of the animals. For example, L-838,417 [34], a partial agonist at α2, α3 and α5 $GABA_A$ receptors and an antagonist at the benzodiazepine-binding site of the α1 subunit, is active against inflammatory and neuropathic hyperalgesia in rats and reduces hyperalgesia-associated brain activation in rat functional magnetic resonance imaging (fMRI) experiments [30]. SL651498 [35], which shows functional selectivity for $GABA_A$ receptor α2 and α3 subunits, reduces both formalin-evoked and electrically evoked nociceptive flexor responses [31,36]. A rather extensive assessment has been performed for NS11394 [37], a recently developed subtype-selective agent. NS11394 possesses an intrinsic activity profile of $\alpha5 > \alpha3 > \alpha2 >> \alpha1$. This compound exerted profound antihyperalgesic effects in several mouse and rat pain models but, as expected, no general analgesic properties in naive rodents [38] and no sedation at doses of up to 30 mg/kg [38].

Although these experiments clearly confirm that subtype-selective benzodiazepine-site ligands exert antihyperalgesic activity after systemic administration, the limited

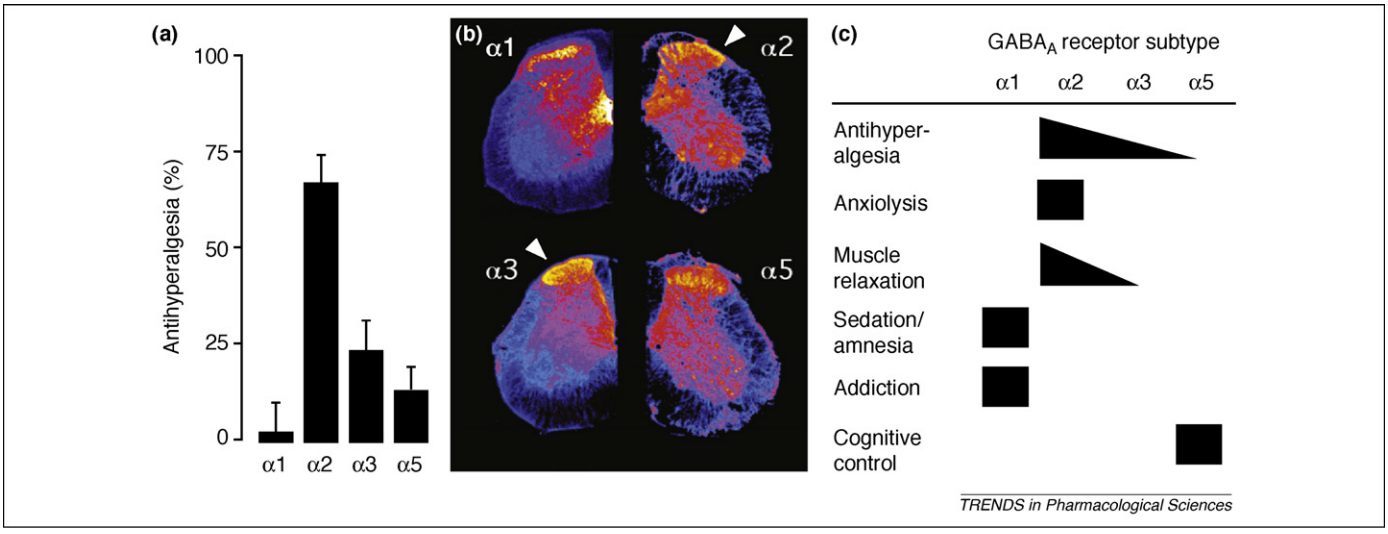

Figure 2. GABA$_A$ receptor subtypes and spinal antihyperalgesia. **(a)** Contribution of different GABA$_A$ receptor α subunits to antihyperalgesia by spinal diazepam in inflammatory heat hyperalgesia (data derived from Ref. [30]). **(b)** Distribution of benzodiazepine-sensitive GABA$_A$ receptor α subunits in the mouse spinal cord (courtesy of Jolly Paul and Jean-Marc Fritschy). Note the presence of α2 and α3 in the most superficial layers of the dorsal horn, where the nociceptive afferents terminate (arrow heads). **(c)** Relative relevance of different GABA$_A$ receptor α subunits to antihyperalgesia and other desired or undesired *in vivo* effects of diazepam. Data on antihyperalgesia, anxiolysis, muscle relaxation and sedation/amnesia are derived from studies in GABA$_A$ receptor mutant mice [28–30]. Data on cognitive control are from Ref. [40], which employed an α5-selective inverse agonist.

Table 1. Pharmacological properties of subtype-selective benzodiazepine-site ligands tested in pain studies

Compound	Intrinsic activity at α1 / α2 / α3 / α5	Major *in vivo* effects	Analgesic or antihyperalgesic effects
L-838,417[a]	0.01 / 0.43 / 0.43 / 0.38[b] [34]	Anxiolysis (rat elevated plus maze ≥ 1 mg/kg, p.o.) No sedation (mouse locomotor activity ≤ 30 mg/kg, i.p.) No motor impairment (mouse rotarod ≤ 30 mg/kg, p.o.) [34]	Antihyperalgesic in rats in inflammatory and neuropathic pain models (0.1–10 mg/kg, i.p., p.o.) but no effect on acute nociceptive pain [30]
SL651498	0.45 / >1 / 0.83 / 0.50[c] [35]	Anxiolysis (rat elevated plus maze, ≥ 1 mg/kg, i.p.) Muscle relaxation (mouse horizontal wire ≥ 30 mg/kg, p.o.) No sedation (mouse locomotor activity, ≤ 10 mg/kg, i.p.) [35]	Antinociceptive in the mouse formalin test (10 mg/kg, p.o.) [31] Reduced electrically induced flexor reflexes in rats (3 mg/kg, i.p.) [36]
NS11394	0.078 / 0.26 / 0.52 / 0.78[d] [37]	Anxiolysis (mouse four plate test, ≥ 0.3 mg/kg, p.o.) [37] No sedation (mouse locomotor activity, ≤ 100 mg/kg, p.o.) [38] No motor impairment (rat rotarod, < 120 mg/kg, p.o.) [38]	No effect on acute nociceptive pain in the hot plate test (≤ 30 mg/kg, p.o.) [38] Antinociceptive in phase II of the rat formalin test (≥ 3 mg/kg, p.o.), increased weight bearing on inflamed paw (≥ 1 mg/kg, p.o.), reduced neuropathic pain (≥ 3 mg/kg, p.o.) [38]

Abbreviations: i.p., intraperitoneally injected; p.o., per os.
[a]Note poor pharmacokinetics of L-838,417 in mice [47].
[b]Percent potentiation of I_{GABA} at EC20 of GABA.
[c]Relative to zolpidem (α1) or diazepam (α2, α3 and α5).
[d]Relative to diazepam.

number of subtype-selective compounds tested in rodent models of pain still does not allow a firm conclusion as to whether or not the subunit specificity obtained from mutant mouse studies can be fully replicated with subtype-selective compounds. For example, NS11394, which possesses the highest intrinsic activity at α5 subunits, showed pronounced antihyperalgesic activity although data from the point-mutated mice indicated that this subunit contributes only marginally to spinal antihyperalgesia. The dose of NS11394 required for significant antihyperalgesia (1–3 mg/kg) [38] yields an *in vivo* receptor occupancy of 70–90% in mice [37], which – given the rather high intrinsic activity of 25 and 50% at α2 and α3 [37], respectively – would also be consistent with action via α2 and α3 GABA$_A$ receptors. Other obvious confounding factors on the side of subtype-selective agents include, in particular, pharmacologically active metabolites, which have not been studied in sufficient detail for most subtype-selective compounds. However, the mutant mouse approach might also have intrinsic limitations with regard to receptor occupancy. When α2, α3 or α5 point-mutated animals are tested for loss of a pharmacological effect, a low (sub-sedative) dose of systemic diazepam is often used to avoid confounding sedation. By contrast, when a

non-sedative subtype-selective drug is given, it can be tested at high doses, reaching up to 100% occupancy at a particular receptor. Under these conditions, a contribution from a previously unexpected receptor subtype might become apparent that had not been detected in the mutants, in which diazepam had been tested at low receptor occupancy.

Undesired effects are of major concern when the potential use of subtype-selective benzodiazepine-site ligands in pain is considered. Sedative effects, impairment of learning and, in particular, liability to addiction and dependence would preclude the use of classical benzodiazepines in patients with chronic pain. At present, it is still difficult to make firm predictions. Work from mutant mice suggests that not only sedation but also the amnestic properties of diazepam require α1 subunits [28], and studies employing subtype-selective agents largely support these findings [34,37,39]. With regard to hippocampus-dependent learning, work with an inverse agonist (i.e. a ligand which reduces the effect of GABA) at α5 $GABA_A$ receptors [40] and with mutant mice [41] suggests that α5 subunits are involved, but direct evidence for a memory-impairing effect of subtype-selective agents with intrinsic activity at α5 is lacking. High intrinsic activity at the α1 subunit also seems to favor reinforcing (addictive) properties of benzodiazepines. In tests of self-administration and drug discrimination in monkeys, the α1-selective ligands zolpidem and zaleplon acted as strong reinforcers [42], whereas TPA023, which lacks intrinsic activity at α1 receptors [39], failed to do so in both paradigms [42]. SL651498 partially substituted for triazolam, an effect that was blocked by an α1 preferring β-carboline, implicating α1 receptors in the subjective effects of SL651498 [43]. However, L-838,417, which also lacks activity at α1, still displayed low reinforcing activity by self-administration [44]. Physical dependence, which manifests in the generation of withdrawal symptoms upon abrupt discontinuation of the drug or upon application of an antagonist, and tolerance (progressive loss of therapeutic effect during prolonged treatment) have so far not been observed with several of the subtype-selective agents. No withdrawal symptoms (seizures) were evoked upon application of the inverse benzodiazepine agonist FG-7142 after four days of continuous treatment with the subtype-selective agents L-838,417 and SL651498 [45]. Furthermore, unlike morphine, L-838,417 did not lose its antihyperalgesic properties after nine days of chronic treatment [30]. These results strongly suggest that many of the undesired effects of conventional benzodiazepines should be absent in α1-sparing subtype-selective agents (Figure 2c). It should, however, still be kept in mind that, in addition to subunit specificities, other factors such as different intrinsic activities and pharmacokinetic properties can contribute to differences in reinforcing properties and liability to tolerance development and dependence [46].

Concluding remarks

In summary, studies on the neurobiological basis of pain clearly point at a crucial role of inhibitory neurotransmission in spinal nociceptive processing. The findings discussed in this article indicate that α2- and/or α3-containing $GABA_A$ receptors in the spinal dorsal horn serve a key role in this process. Novel compounds activating these receptor subtypes but sparing α1 containing $GABA_A$ receptors would avoid the most relevant unwanted effects of established benzodiazepine-like drugs, namely their sedative, anmestic and addictive properties. Such compounds should, therefore, constitute a new and promising class of antihyperalgesic agents particularly suitable for the treatment of chronic pain syndromes. Future studies with yet to develop subtype-selective agents suitable for the use in human volunteers and patients will have to clarify whether the promising results from rodent pain models can be translated to human pain therapy.

References

1 Zeilhofer, H.U. (2008) Loss of glycinergic and GABAergic inhibition in chronic pain–contributions of inflammation and microglia. *Int. Immunopharmacol.* 8, 182–187
2 Beyer, C. *et al.* (1985) Hyperalgesia induced by altered glycinergic activity at the spinal cord. *Life Sci.* 37, 875–882
3 Yaksh, T.L. (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
4 Homanics, G.E. *et al.* (1997) Mice devoid of γ-aminobutyrate type A receptor β3 subunit have epilepsy, cleft palate, and hypersensitive behavior. *Proc. Natl. Acad. Sci. U. S. A.* 94, 4143–4148
5 Ugarte, S.D. *et al.* (2000) Sensory thresholds and the antinociceptive effects of GABA receptor agonists in mice lacking the β3 subunit of the $GABA_A$ receptor. *Neuroscience* 95, 795–806
6 Zheng, W. *et al.* (2003) Function of γ-aminobutyric acid receptor/channel rho 1 subunits in spinal cord. *J. Biol. Chem.* 278, 48321–48329
7 Coull, J.A. *et al.* (2003) Trans-synaptic shift in anion gradient in spinal lamina I neurons as a mechanism of neuropathic pain. *Nature* 424, 938–942
8 Coull, J.A. *et al.* (2005) BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. *Nature* 438, 1017–1021
9 Moore, K.A. *et al.* (2002) Partial peripheral nerve injury promotes a selective loss of GABAergic inhibition in the superficial dorsal horn of the spinal cord. *J. Neurosci.* 22, 6724–6731
10 Scholz, J. *et al.* (2005) Blocking caspase activity prevents transsynaptic neuronal apoptosis and the loss of inhibition in lamina II of the dorsal horn after peripheral nerve injury. *J. Neurosci.* 25, 7317–7323
11 Polgár, E. *et al.* (2005) Loss of neurons from laminas I-III of the spinal dorsal horn is not required for development of tactile allodynia in the spared nerve injury model of neuropathic pain. *J. Neurosci.* 25, 6658–6666
12 Ahmadi, S. *et al.* (2002) PGE_2 selectively blocks inhibitory glycinergic neurotransmission onto rat superficial dorsal horn neurons. *Nat. Neurosci.* 5, 34–40
13 Harvey, R.J. *et al.* (2004) GlyRα3: an essential target for spinal PGE2-mediated inflammatory pain sensitization. *Science* 304, 884–887
14 Melzack, R. and Wall, P.D. (1965) Pain mechanisms: a new theory. *Science* 150, 971–979
15 Jasmin, L. *et al.* (2004) GABA puts a stop to pain. *Curr. Drug Target. CNS Neurol. Disord.* 3, 487–505
16 Enna, S.J. and McCarson, K.E. (2006) The role of GABA in the mediation and perception of pain. *Adv. Pharmacol.* 54, 1–27
17 Laughlin, T.M. *et al.* (2002) Comparison of antiepileptic drugs tiagabine, lamotrigine, and gabapentin in mouse models of acute, prolonged, and chronic nociception. *J. Pharmacol. Exp. Ther.* 302, 1168–1175
18 Novak, V. *et al.* (2001) Treatment of painful sensory neuropathy with tiagabine: a pilot study. *Clin. Auton. Res.* 11, 357–361
19 Buckett, W.R. (1980) Irreversible inhibitors of GABA transaminase induce antinociceptive effects and potentiate morphine. *Neuropharmacology* 19, 715–722
20 Jasmin, L. *et al.* (2003) Analgesia and hyperalgesia from GABA-mediated modulation of the cerebral cortex. *Nature* 424, 316–320

21 Krogsgaard-Larsen, P. *et al.* (2004) $GABA_A$ agonists and partial agonists: THIP (Gaboxadol) as a non-opioid analgesic and a novel type of hypnotic. *Biochem. Pharmacol.* 68, 1573–1580
22 Chandra, D. *et al.* (2006) $GABA_A$ receptor α4 subunits mediate extrasynaptic inhibition in thalamus and dentate gyrus and the action of gaboxadol. *Proc. Natl. Acad. Sci. U. S. A.* 103, 15230–15235
23 Wafford, K.A. and Ebert, B. (2006) Gaboxadol – a new awakening in sleep. *Curr. Opin. Pharmacol.* 6, 30–36
24 Storustovu, S.I. and Ebert, B. (2006) Pharmacological characterization of agonists at δ-containing $GABA_A$ receptors: Functional selectivity for extrasynaptic receptors is dependent on the absence of γ2. *J. Pharmacol. Exp. Ther.* 316, 1351–1359
25 Kjaer, M. and Nielsen, H. (1983) The analgesic effect of the GABA-agonist THIP in patients with chronic pain of malignant origin. A phase-1-2 study. *Br. J. Clin. Pharmacol.* 16, 477–485
26 Rudolph, U. and Mohler, H. (2004) Analysis of $GABA_A$ receptor function and dissection of the pharmacology of benzodiazepines and general anesthetics through mouse genetics. *Annu. Rev. Pharmacol. Toxicol.* 44, 475–498
27 Benson, J.A. *et al.* (1998) Pharmacology of recombinant γ-aminobutyric acidA receptors rendered diazepam-insensitive by point-mutated α-subunits. *FEBS Lett.* 431, 400–404
28 Rudolph, U. *et al.* (1999) Benzodiazepine actions mediated by specific γ-aminobutyric $acid_A$ receptor subtypes. *Nature* 401, 796–800
29 Low, K. *et al.* (2000) Molecular and neuronal substrate for the selective attenuation of anxiety. *Science* 290, 131–134
30 Knabl, J. *et al.* (2008) Reversal of pathological pain through specific spinal $GABA_A$ receptor subtypes. *Nature* 451, 330–334
31 Knabl, J. *et al.* (2009) Genuine antihyperalgesia by systemic diazepam revealed by experiments in $GABA_A$ receptor point-mutated mice. *Pain* 141, 233–238
32 Keller, A.F. *et al.* (2001) Region-specific developmental specialization of GABA-glycine cosynapses in laminas I-II of the rat spinal dorsal horn. *J. Neurosci.* 21, 7871–7880
33 Baccei, M.L. and Fitzgerald, M. (2004) Development of GABAergic and glycinergic transmission in the neonatal rat dorsal horn. *J. Neurosci.* 24, 4749–4757
34 McKernan, R.M. *et al.* (2000) Sedative but not anxiolytic properties of benzodiazepines are mediated by the $GABA_A$ receptor α1 subtype. *Nat. Neurosci.* 3, 587–592
35 Griebel, G. *et al.* (2001) SL651498: an anxioselective compound with functional selectivity for α2- and α3-containing γ-aminobutyric $acid_A$ ($GABA_A$) receptors. *J. Pharmacol. Exp. Ther.* 298, 753–768
36 Griebel, G. *et al.* (2003) SL651498, a $GABA_A$ receptor agonist with subtype-seletive efficacy, as a potential treatment for geeralized anxiety disorder and muscle spasm. *CNS Drug Rev.* 9, 3–20
37 Mirza, N.R. *et al.* (2008) NS11394 [3′-[5-(1-hydroxy-1-methyl-ethyl)-benzoimidazol-1-yl]-biphenyl-2-carbonitrile], a unique subtype-selective $GABA_A$ receptor positive allosteric modulator: *in vitro* actions, pharmacokinetic properties and *in vivo* anxiolytic efficacy. *J. Pharmacol. Exp. Ther.* 327, 954–968
38 Munro, G. *et al.* (2008) Comparison of the novel subtype-selective $GABA_A$ receptor-positive allosteric modulator NS11394 [3′-[5-(1-hydroxy-1-methyl-ethyl)-benzoimidazol-1-yl]-biphenyl-2-carbonitrile] with diazepam, zolpidem, bretazenil, and gaboxadol in rat models of inflammatory and neuropathic pain. *J. Pharmacol. Exp. Ther.* 327, 969–981
39 Atack, J.R. *et al.* (2006) TPA023 [7-(1,1-dimethylethyl)-6-(2-ethyl-2H-1,2,4-triazol-3-ylmethoxy)-3-(2-fluorophenyl)-1,2,4-triazolo[4,3-b]pyridazine], an agonist selective for α2- and α3-containing $GABA_A$ receptors, is a nonsedating anxiolytic in rodents and primates. *J. Pharmacol. Ther.* 206, 410–422
40 Dawson, G.R. *et al.* (2006) An inverse agonist selective for α5 subunit-containing $GABA_A$ receptors enhances cognition. *J. Pharmacol. Exp. Ther.* 316, 1335–1345
41 Crestani, F. *et al.* (2002) Trace fear conditioning involves hippocampal α5 $GABA_A$ receptors. *Proc. Natl. Acad. Sci. U. S. A.* 99, 8980–8985
42 Ator, N. (2005) Contributions of $GABA_A$ receptor subtype selectivity to abuse liability and dependence potential of pharmacological treatments for anxiety and sleep disorders. *CNS Spectr.* 10, 31–39
43 Licata, S.C. *et al.* (2005) Contribution of $GABA_A$ receptor subtypes to the anxiolytic-like, motor, and discriminative stimulus effects of benzodiazepines: studies with the functionally selective ligand SL651498 [6-fluoro-9-methyl-2-phenyl-4-(pyrrolidin-1-yl-carbonyl)-2,9-dihydro-1H-pyridol[3,4-b]indol-1-one]. *J. Pharmacol. Exp. Ther.* 313, 1118–1125
44 Rowlett, J.K. *et al.* (2005) Different $GABA_A$ receptor subtypes mediate the anxiolytic, abuse-related, and motor effects of benzodiazepine-like drugs in primates. *Proc. Natl. Acad. Sci. U. S. A.* 102, 915–920
45 Mirza, N.R. and Nielsen, E.O. (2006) Do subtype-selective γ-aminobutyric acid A receptor modulators have a reduced propensity to induce physical dependence in mice? *J. Pharmacol. Exp. Ther.* 316, 1378–1385
46 Busto, U. *et al.* (1989) Clinical pharmacokinetics of non-opiate abused drugs. *Clin. Pharmacokinet.* 16, 1–26
47 Scott-Stevens, P. *et al.* (2005) Rodent pharmacokinetics and receptor occupancy of the $GABA_A$ receptor subtype selective benzodiazepine site ligand L-838417. *Biopharm. Drug Dispos.* 26, 13–20
48 Baba, H. *et al.* (2003) Removal of GABAergic inhibition facilitates polysynaptic A fiber-mediated excitatory transmission to the superficial spinal dorsal horn. *Mol. Cell. Neurosci.* 24, 818–830
49 Torsney, C. and MacDermott, A.B. (2006) Disinhibition opens the gate to pathological pain signaling in superficial neurokinin 1 receptor-expressing neurons in rat spinal cord. *J. Neurosci.* 26, 1833–1843
50 Antal, M. *et al.* (1996) Direct evidence of an extensive GABAergic innervation of the spinal dorsal horn by fibres descending from the rostral ventromedial medulla. *Neuroscience* 73, 509–518
51 Kato, G. *et al.* (2006) Direct GABAergic and glycinergic inhibition of the substantia gelatinosa from the rostral ventromedial medulla revealed by *in vivo* patch-clamp analysis in rats. *J. Neurosci.* 26, 1787–1794