Available online at www.sciencedirect.com

SCIENCE DIRECT

European Journal of Pharmacology 516 (2005) 131 – 138

www.elsevier.com/locate/ejphar

Centrally-mediated antinociceptive actions of $GABA_A$ receptor agonists in the rat spared nerve injury model of neuropathic pain

Frederik Rode[a,*], Dorthe G. Jensen[a], Gordon Blackburn-Munro[b], Ole J. Bjerrum[a]

[a]*Danish University of Pharmaceutical Sciences, Department of Pharmacology, Jagtvej 120, Copenhagen, Denmark*
[b]*Department of Pharmacology, Neurosearch A/S, Pederstrupvej 93, Ballerup, DK-2750, Denmark*

Received 27 January 2005; received in revised form 18 April 2005; accepted 22 April 2005

Abstract

Gamma aminobutyric acid (GABA) plays a major role in the central hyperexcitabilty associated with nerve damage. The precise antinociceptive actions mediated by $GABA_A$ receptor agonists remain unclear as previous studies have shown mixed results in neuropathic pain models. Thus, various drugs which modulate $GABA_A$ receptor function were tested in the rat spared nerve injury (SNI) model of neuropathic pain. The selective $GABA_A$ receptor agonist gaboxadol dose-dependently (6 and 15 mg/kg, s.c.) reversed hindpaw mechanical allodynia and hyperalgesia for at least 150 min after administration. The $GABA_A$ receptor agonist muscimol (0.02–2 mg/kg, s.c.) also dose-dependently reversed mechanical allodynia, although the maximal effect achieved was less than that observed for gaboxadol. Mechanical hyperalgesia was attenuated only by the highest dose of muscimol. In contrast, the selective $GABA_A$ receptor agonist isoguvacine (20 mg/kg, s.c.) which has poor central nervous system penetration, and the benzodiazepine-site ligand zolpidem (20 mg/kg, s.c.) were ineffective against either nociceptive behaviour. In the rotarod test, both gaboxadol (15 mg/kg) and zolpidem impaired motor function for at least 60 min after injection; muscimol (2 mg/kg) and gaboxadol (6 mg/kg) were ineffective. Importantly, the ataxic effects induced by gaboxadol resolved 1–2 h after administration, a time point where clear antiallodynic and antihyperalgesic actions still occurred. Thus, systemic administration of blood–brain penetratable selective $GABA_A$ receptor agonists attenuate nociceptive behaviours in the SNI rat model of neuropathic pain that can be considered to occur independently of other effects on motor function.

Keywords: Allodynia; Gaboxadol; $GABA_A$ receptor; Hyperalgesia; Isoguvacine; Zolpidem

1. Introduction

Neuropathic pain can arise from damage to nerves within the peripheral or central nervous systems, either as a direct result of physical injury or as a consequence of metabolic dysfunction or viral infection. It can be difficult to treat with typical analgesics, an implication of which is that two-thirds of neuropathic pain patients do not experience sufficient pain relief (Jensen and Baron, 2003). Quality and quantity of sleep are also affected (Nicholson and Verma, 2004). This latter feature when taken together with deleterious sensory deficits can mean that neuropathic pain patients have an increased propensity for developing psychiatric disorders. There is now substantial evidence to suggest that anti-depressants and antiepileptics may improve signs and symptoms of neuropathic pain. Nevertheless, neither drug class offers broad-spectrum pain relief (Dworkin et al., 2003). Thus, other drug classes with alternative mechanisms of action may help to resolve unmet needs in neuropathic pain patients (Harden and Cohen, 2003).

Gamma aminobutyric acid (GABA) is the principal inhibitory neurotransmitter within the superficial laminae of the spinal dorsal horn, as indicated from animal studies which have reported enhanced responsiveness to tactile and chemical stimulation after intrathecal administration of the $GABA_A$ receptor antagonist bicuculline (Kaneko and Hammond, 1997; Malan et al., 2002). A more direct involvement of $GABA_A$ receptors in persistent pain states

* Corresponding author. Tel.: +4535306515; fax: +4535306020.
E-mail address: FR@dfuni.dk (F. Rode).

0014-2999/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2005.04.034

is indicated by the attenuating actions of the $GABA_A$ receptor agonists muscimol and isoguvacine on second phase flinching behaviours in the rat formalin test (Kaneko and Hammond, 1997). In addition, it has been shown that antagonism of $GABA_A$ receptors enhances spinal cord central sensitisation (Sivilotti and Woolf, 1994). In turn, central sensitisation is thought to underlie the behavioural manifestation of sensory deficits such as allodynia and hyperalgesia in animal models of neuropathic pain (Blackburn-Munro, 2004). Accordingly, the spinal administration of $GABA_A$ receptor agonists such as muscimol and isoguvacine, has been reported to attenuate behavioural allodynia and hyperalgesia in multiple animal models of neuropathic pain (Malan et al., 2002; Cui et al., 1996). However, inconsistencies are apparent, since muscimol has been reported to be ineffective in attenuating pain associated with photochemically-induced sciatic nerve injury and photochemically-induced ischaemic spinal cord injury (Hao et al., 1991; Hao et al., 1999).

The selective $GABA_A$ receptor agonist gaboxadol [4,5,6,7-tetrahydroisoxazolo(5,4-c)pyridin-3-ol; THIP] is a conformationally restricted analogue of muscimol with anticonvulsant properties, which is currently under registration for the treatment of insomnia (Frolund et al., 2002). Gaboxadol has also been anecdotally reported to attenuate nociceptive pain in both animals and humans (Krogsgaard-Larsen et al., 1997; Frolund et al., 2002). Gaboxadol has greater affinity for central $GABA_A$ receptors than muscimol and is better tolerated after systemic administration (Krogsgaard-Larsen et al., 2004). Furthermore, unlike the benzodiazepine-site ligand zolpidem, which acts to positively modulate $GABA_A$ receptor function, gaboxadol effectively promotes sleep without inducing tolerance after chronic treatment in rats (Lu et al., 2003).

Given that gaboxadol appears to have a more favourable side-effect profile than other $GABA_A$ receptor agonists (Krogsgaard-Larsen et al., 2004), we have tested for antinociceptive effects of systemically administered gaboxadol in the rat spared nerve injury model of neuropathic pain. For comparison we also tested morphine, muscimol, isoguvacine (which has poorer central nervous system penetration than muscimol or gaboxadol; Krogsgaard-Larsen et al., 1981) and zolpidem in the same model.

2. Methods

Adult male Sprague–Dawley rats (Taconic, Denmark) weighing 250 g on the day of surgery were used in this study except where mentioned. They were housed in groups of five in cages containing soft bedding. Food and water were available ad libitum and the light–dark-cycle was 12:12 h. The animals were habituated to their surroundings for 14 days prior to surgery. All experiments were performed according to the Danish Committee for Experiments on Animals and the ethical guidelines of the International Association of the Study of Pain (Zimmermann, 1983).

2.1. Surgery

The method of inducing spared nerve injury (SNI) has been described in detail previously (Decosterd and Woolf, 2000). Rats were anaesthetised with a subcutaneous (s.c.) mixture of fentanyl (5 mg/kg) and midazolam (2.5 mg/kg). The skin of the lateral left thigh was incised and the overlying musculature separated to expose the sciatic nerve and its three terminal branches; the sural, tibial and common peroneal nerves. The tibial and common peroneal nerves were ligated with 5/0 silk and sectioned distal to the ligation. Any stretching or contact with the intact sural nerve was avoided. The muscle was then closed in layers and the skin sutured together.

2.2. Behavioural testing

The testing for the presence of neuropathic pain behaviours was performed in the same room in which the animals were routinely housed. Animals were tested before surgery, on the third day after surgery and a further four times over the following two weeks after injury. On the day of testing, SNI rats were placed in individual plexiglass compartments with a metal grid floor. The testing compartments were elevated to provide the operator with easy access to the plantar surface of the rat's hindpaws.

On the day of drug testing a 30 min adaptation period prior to baseline testing was allowed. All drug testing in SNI rats was performed blind by the observer, using a semi-randomized cross-over paradigm. Only those animals (20 in total) with distinct neuropathic behaviour were included in the study, and all SNI rats received more than one dose of drug and were assigned to more than one drug treatment. A minimal period of 3–4 days was allowed for drug washout, and the studies were conducted from 4 to 12 weeks after surgery. On the day of testing, after a baseline measurement was made prior to drug injection, the effect of the drug on the behavioural response was monitored at 30-min intervals for up to 150 min post-injection.

2.2.1. Mechanical allodynia

A set of von Frey monofilaments (0.008 g–100 g, Stoelting, IL, USA) was used to test the mechanical withdrawal threshold of the hindpaws. The monofilaments or hairs were applied to the lateral plantar surface of the hindpaw (Decosterd and Woolf, 2000). The hairs were applied with increasing force, starting from below the withdrawal threshold until the rat withdrew the paw. The force in *g* of the von Frey hair able to cause 3 withdrawals after 5 consecutive stimulations (separated by 1–2 s) was taken as the withdrawal threshold. Lifting of the paw due to normal locomotor activity was ignored (Hao et al., 1999).

2.2.2. Mechanical hyperalgesia

A new safety pin (0.7 mm diameter and 5 cm long) was applied to the allodynic area of the ipsilateral hindpaw of the rat. The pin was routinely applied with a force sufficient to produce a reflex withdrawal response in non-operated animals, but at an intensity insufficient to penetrate the skin. The duration of the paw withdrawal was measured with a stopwatch. Prior to surgery, the withdrawal duration was too short to time accurately and was set to an arbitrarily minimal time of 0.5 s. A cut-off time of 15 s was applied for the injured hindpaw after surgery (Erichsen and Blackburn-Munro, 2002; Tal and Bennett, 1994).

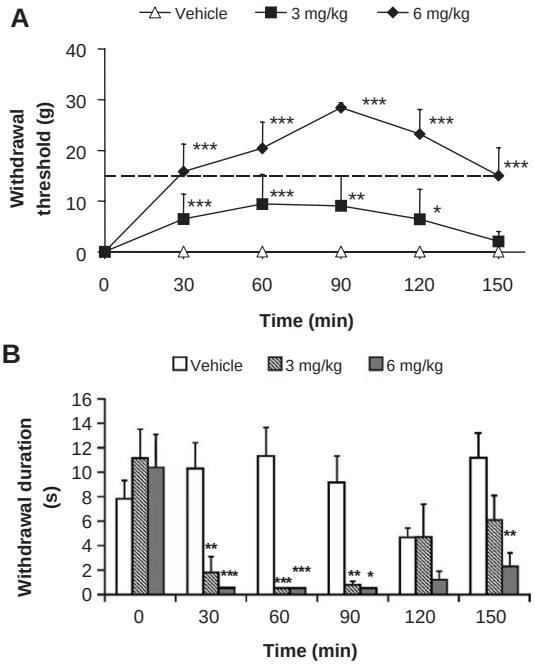

Fig. 1. Effect of the μ-opioid receptor agonist morphine on nociceptive responses in SNI rats. Morphine (3 and 6 mg/kg, s.c.) or vehicle was administered immediately after the baseline response (time 0) had been obtained and the time course of drug actions followed. (A) Paw withdrawal threshold (g) in response to von Frey hair stimulation of the injured hindpaw (as an indication of mechanical allodynia) was dose-dependently reversed by morphine. (B) Paw withdrawal duration (s) in response to pin prick stimulation of the injured hindpaw (as an indication of mechanical hyperalgesia) was dose-dependently attenuated by morphine. Dashed line represents pre-surgery value. All groups, $n=6$ animals. Data are presented as mean±S.E.M. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ vs. vehicle (two-way ANOVA followed by Bonferroni's *t*-test).

2.2.3. Rotarod test

In normal, uninjured rats (Harlan Scandinavia, Denmark; 250–300 g) changes in motor performance after drug administration were measured using the rotarod test (Blackburn-Munro et al., 2004). Rats were placed on the rotating rod (Ugo Basile, Italy) and required to walk against the motion as the speed was accelerated from 3 rpm to 30 rpm, with the time spent on the rod measured for up to 180 s. Rats received two training trials (separated by 3–4 h) the day prior to drug testing for acclimitisation purposes. On the day of testing after a baseline response had been obtained, they were subsequently administered with drug or vehicle according to the experimental paradigm, and the time course of motor performance followed every 30 min for up to 150 min.

2.3. Drugs

Morphine hydrochloride was purchased from Nordichem, Denmark. Gaboxadol HCl and muscimol were synthesized within the Department of Medicinal Chemistry at The Danish University of Pharmaceutical Sciences by P. Krogsgaard Larsen and B. Frølund, respectively. Zolpidem and isoguvacine were purchased from Sigma-Aldrich, Denmark. All drugs were dissolved in isotonic saline and administered s.c. in a dosing volume of 1 ml/kg.

2.4. Statistics

Statistical comparisons of paw withdrawal threshold values in response to von Frey stimulation were made on natural log transformed data. This was undertaken to make the errors of the smaller and larger values comparable for analysis of variance (ANOVA), as the errors become additive after the transformation. Statistical comparisons of rotarod and pin prick values were undertaken on the raw data since there was no systematic error difference between higher and lower values. Two-way ANOVA followed by Bonferroni's test was used as appropriate. $P<0.05$ was considered significant in all cases.

3. Results

3.1. General observations

The body weight of SNI rats decreased the first week after surgery, but no further impact on weight gain compared to non-injured age-matched control animals was noted. Although no autotomy was observed the rats did display a flexed paw. In addition, the majority of rats attempted to avoid contact between the allodynic areas of the injured paw and regulated their weight distribution accordingly towards the non-injured, contralateral hindpaw. The most allodynic area of the hindpaw was the hairy

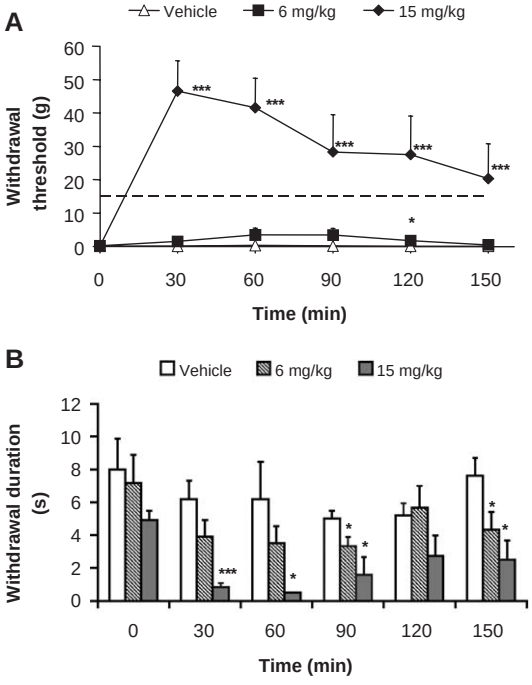

Fig. 2. Effect of the selective $GABA_A$ receptor agonist gaboxadol on nociceptive responses in SNI rats. Gaboxadol (6 and 15 mg/kg, s.c.) or vehicle was administered immediately after the baseline response had been obtained and the time course of drug actions followed. (A) Paw withdrawal threshold (g) in response to von Frey hair stimulation of the injured hindpaw was dose-dependently reversed by gaboxadol. (B) Paw withdrawal duration (s) in response to pin prick stimulation of the injured was dose-dependently attenuated by gaboxadol. Dashed line represents pre-surgery value. All groups, $n=7$ animals. Data are presented as mean±S.E.M. $^{*}P<0.05$, $^{***}P<0.001$ vs. vehicle (two-way ANOVA followed by Bonferroni's *t*-test).

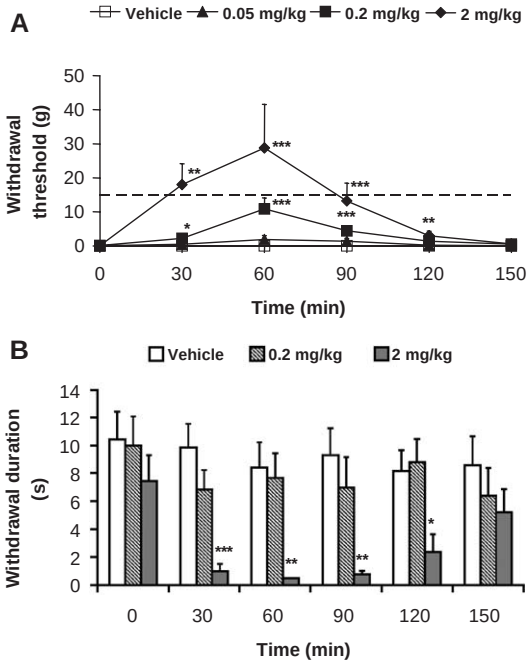

Fig. 3. Effect of the selective $GABA_A$ receptor agonist muscimol on nociceptive responses in SNI rats. Muscimol (0.05–2 mg/kg, s.c.) or vehicle was administered immediately after the baseline response had been obtained and the time course of drug actions followed. (A) The paw withdrawal threshold (g) in response to von Frey hair stimulation of the injured hindpaw was dose-dependently reversed by muscimol. (B) The paw withdrawal duration (s) in response to pin prick stimulation of the injured was attenuated by muscimol only at the highest dose tested. Dashed line represents pre-surgery value. All groups, $n=6–7$ animals. Data are presented as mean±S.E.M. *$P<0.05$, **$P<0.01$, ***$P<0.001$ vs. vehicle (two-way ANOVA followed by Bonferroni's t-test).

lateral plantar skin, which started as a thin line from the tip of digit 5 and broadened towards the back of the foot and ankle. Although contralateral hypersensitivity to von Frey hair stimulation appeared to increase after injury, this could not be consistently measured, due to the rats displaying generalized escape behaviour to the stimulus rather than initiating a reflex hindpaw withdrawal as was observed for the ipsilateral hindpaw. Mechanical hyperalgesia was measured over the entire hindpaw. Prior to surgery the paw withdrawal threshold in response to von Frey hair stimulation was 17±10 g ($n=15$) and paw withdrawal duration in response to pin prick stimulation was always < 0.5 s. Four days after surgery the paw withdrawal threshold fell to 0.98±0.2 g ($P<0.001$) in SNI rats, whereas the paw withdrawal duration had increased to 6±2.1 s, indicative of mechanical hyperalgesia. Baseline hindpaw mechanical allodynia remained at a similar level up to 3 months after surgery.

3.2. Drug testing in SNI rats

3.2.1. Effects of morphine

Administration of morphine (3 and 6 mg/kg, s.c.) to SNI rats produced a dose-dependent reversal of hindpaw mechanical allodynia [time effect $F_{(2,78)}=98.5$, $P<0.0001$] and mechanical hyperalgesia [time effect $F_{(2,78)}=25.33$, $P<0.0001$; Fig. 1]. The onset of antiallodynic and antihyperalgesic actions were observed from 30 min after injection for both doses of morphine tested. Injection of morphine at 6 mg/kg produced a particularly marked increase in the paw withdrawal threshold in response to von Frey hair stimulation at 90 min post-injection (28.4±1.0 g, $P<0.001$ vs. baseline). The antiallodynic and antihyperalgesic actions of morphine remained significantly different from baseline for at least 150 min after injection. We have previously tested morphine (3 and 6 mg/kg) in the rotarod test after s.c. administration and observed no effect on motor function (Erichsen and Blackburn-Munro, 2002).

3.2.2. Effects of gabaxadol

Subcutaneous administration of gaboxadol (6 and 15 mg/kg) reversed hindpaw mechanical allodynia [time effect $F_{(5,102)}=5.66$, $P=0.0001$] in SNI rats (Fig. 2A). The onset of antiallodynia was observed at 30 min after injection and remained significantly different from baseline throughout the 150 min duration of the experiment (all time points, $P<0.001$). Furthermore, the increase in paw withdrawal threshold after 15 mg/kg gaboxadol in SNI rats exceeded the pre-surgery threshold level indicative of a general analgesic action. Hindpaw mechanical hyperalgesia was dose-dependently attenuated by gaboxadol [time effect $F_{(5,96)}=6{,}52$, $P<0.002$] in SNI rats (Fig. 2B). The antihyperalgesic actions of gaboxadol were still significantly different from baseline 150 min after injection ($P<0.05$ vs. baseline).

In the rotarod test, gaboxadol (15 mg/kg) produced significant motor impairment in normal uninjured rats at 30 and 60 min after injection compared with baseline and vehicle-treated rats ($P<0.001$; Fig. 5). However, the ataxia had resolved by 120 min after administration. The lower dose of gaboxadol (6 mg/kg) had no effect on motor function.

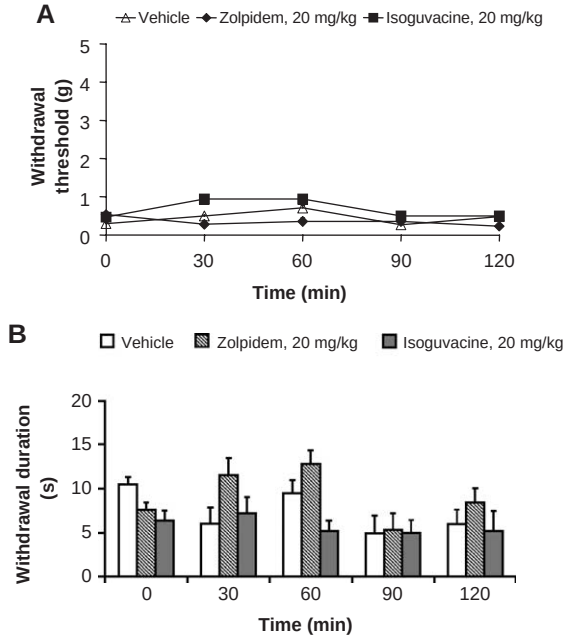

Fig. 4. Effect of the selective $GABA_A$ receptor agonist isoguvacine and the benzodiazepine-site ligand zolpidem on nociceptive responses in SNI rats. Isoguvacine (20 mg/kg, s.c.), zolpidem (20 mg/kg, s.c.) or vehicle was administered immediately after the baseline response had been obtained and the time course of drug actions followed. (A) Paw withdrawal threshold (g) and (B) paw withdrawal duration (s) of the injured hindpaw were unaffected by administration of either isoguvacine or zolpidem. Dashed line represents pre-surgery value. All groups, $n=7$ animals. Data are presented as mean±S.E.M.

3.2.3. Effects of muscimol

Muscimol (0.05–2 mg/kg, s.c.) administration produced a dose-dependent reversal of mechanical allodynia in SNI rats [time effect $F_{(2.90)}=53.53$, $P<0.0001$; Fig. 3A). The associated increase in paw withdrawal threshold was observed as early as 30 min after administration, and reached a maximal value of 28.8±12 g 60 min after administration for the highest dose of muscimol tested ($P<0.001$ vs. baseline). The antiallodynic actions of muscimol remained until 120 min post-injection. Subcutaneous administration of muscimol also reversed hindpaw mechanical hyperalgesia [time effect $F_{(2,102)}=25.26$, $P<0.0001$] in SNI rats (Fig. 3B). However, only the highest dose of muscimol tested (2 mg/kg) attenuated the paw withdrawal duration in response to pin prick stimulation in SNI rats. In the rotarod test, no motor impairment was observed in normal, unoperated rats after s.c. administration of 2 mg/kg muscimol (Fig. 5).

3.2.4. Effects of isoguvacine and zolpidem

In SNI rats, administration of either isoguvacine or zolpidem (both 20 mg/kg, s.c.) failed to affect the paw withdrawal threshold in response to von Frey hair stimulation or the paw withdrawal duration in response to pin prick stimulation (Fig. 4). Administration of zolpidem significantly impaired motor function at 30 and 60 min after administration (both time points $P<0.001$; Fig. 5). No further ataxia was observed for zolpidem 120 min post-injection.

4. Discussion

The present results confirm previous findings that have reported antinociceptive effects for the $GABA_A$ receptor agonist muscimol in animal models of peripheral nerve injury. We have also shown for the first time that systemic administration of the selective $GABA_A$ receptor agonist gaboxadol attenuates both mechanical allodynia and hyperalgesia in SNI rats, at time points where no impairment of motor function was present. These effects appear to have been mediated by actions on centrally located $GABA_A$ receptors, since systemic administration of isoguvacine which has poor central nervous system penetration in comparison with gaboxadol and muscimol failed to affect mechanical hypersensitivity in SNI rats.

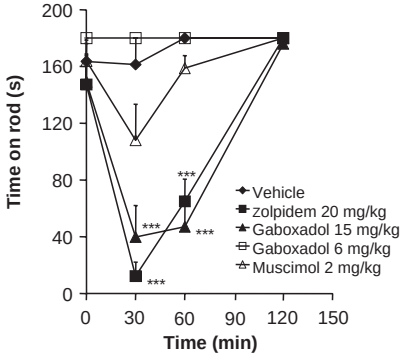

Fig. 5. Effect of $GABA_A$ receptor agonists on motor function in normal, uninjured rats. Gaboxadol (5 and 16 mg/kg), muscimol (2 mg/kg), zolpidem (20 mg/kg) or vehicle were administered s.c. immediately after the baseline response was obtained and the time course of drug actions followed. The time spent on the rotarod (s) was significantly attenuated for up to 1 h after injection by both the highest dose of gaboxadol tested and zolpidem. All groups, $n=7–8$ animals. Data are presented as mean±S.E.M. ***$P<0.001$ vs. vehicle (two-way ANOVA followed by Bonferroni's *t*-test).

4.1. General observations

Rats with spared nerve injury developed pronounced hypersensitivity to von Frey and pin prick stimulation of the injured hindpaw in accordance with previous observations for this model (Decosterd and Woolf, 2000; Decosterd et al., 2004; Erichsen and Blackburn-Munro, 2002). Pronounced mechanical allodynia of the injured hindpaw was still apparent up to 90 days after surgery. Interestingly, the area of the paw innervated by the saphenous nerve was found to be as sensitive to von Frey hair stimulation as compared to the spared sural nerve area a finding that has also been reported in the chronic constriction injury model (CCI; Tal and Bennett, 1994). Furthermore, SNI rats responded to pin prick stimulation when the stimulus was applied to areas of the plantar hindpaw where mechanical allodynia was absent. This corresponds well to the clinical situation, where the hyperalgesic area is often larger than the allodynic area (Gottrup et al., 1998; Raja et al., 1999). As a prelude to testing $GABA_A$ receptor agonists in the SNI rat model we tested for antiallodynic and antihyperalgesic effects of the μ-opioid receptor agonist morphine. Overall, the current findings agree with those previously reported for morphine in SNI rats (Decosterd et al., 2004; Erichsen and Blackburn-Munro, 2002). However, our results indicate that morphine appeared both more potent and efficacious in reversing mechanical allodynia and hyperalgesia. Although adult male Sprague–Dawley rats were used in both studies they were supplied by different vendors. Substrain differences in opioid sensitivity in rats supplied by different vendors have been reported previously, most likely as a result of differential opioid metabolism (Bulka and Wiesenfeld-Hallin, 2003; Bulka et al., 2004).

4.2. GABA and spinal disinhibition after nerve injury

The initiation and maintenance of neuropathic pain involves a complex interplay between excitatory and inhibitory influences arising from within the spinal dorsal horn and supraspinal sites involved in nociceptive processing (Woolf and Salter, 2000). Various lines of evidence suggest that the inhibitory actions of GABA may be diminished after nerve injury. Immunoreactivity for GABA and its synthesizing enzyme glutamate decarboxylase within the dorsal horn appears to be reduced after peripheral nerve injury (Eaton et al., 1998). However, this finding has recently been questioned by Polgar et al. (2004) who failed to observe any changes in GABA cell number or neuropil staining intensity in the CCI model of neuropathic pain. Nevertheless, the incidence, magnitude

and duration of primary afferent evoked GABA-mediated inhibitory postsynaptic currents in lamina II neurones is reduced after both CCI and SNI, presumably as a consequence of attenuated presynaptic GABA release (Moore et al., 2002). Crucially, behavioural studies indicate that intrathecal administration of both $GABA_A$ and $GABA_B$ receptor agonists attenuate mechanical allodynia and thermal hyperalgesia in animal models of peripheral nerve injury (Malan et al., 2002).

Our results support and extend the above findings. Thus, muscimol was shown to effectively reverse mechanical hypersensitivity in SNI rats after systemic administration, while no motor impairment was observed in control rats at doses that produced antinociception. Similarly, systemic administration of gaboxadol produced a profound reversal of mechanical hypersensitivity in SNI rats. Indeed, the paw withdrawal duration was raised above that measured prior to surgery throughout the 150 min experimental duration. Although marked motor impairment was observed after administration of gaboxadol (15 mg/kg), crucially, this potentially confounding issue had dissipated between 1 and 2 h after administration. Thus, we are confident that gaboxadol specifically attenuated injury-induced mechanical hypersensitivity in SNI rats. Furthermore, the benzodiazepine-site ligand zolpidem failed to attenuate pain behaviours in SNI rats despite producing marked motor impairment with a similar temporal profile to that observed for gaboxadol. This further emphasizes that disruption of motor function per se does not necessarily correlate with the inability of the rat to initiate a withdrawal reflex to experimenter-evoked noxious stimulation.

4.3. $GABA_A$ receptor function and correlation with antinociception after nerve injury

$GABA_A$ receptors are highly diverse and at least 20 different subunits have been identified. The pentameric structure of the $GABA_A$ receptor is usually composed of two α subunits, two β subunits and one γ, ε or δ subunit (Mehta and Ticku, 1999; Sieghart et al., 1999). The binding site for GABA and $GABA_A$ receptor agonists lies at the interface between the α and β subunits, whilst the benzodiazepine-site lies between the α and the γ subunit (Frolund et al., 2002). Agonist-induced sedation/hypnosis is mediated primarily by $GABA_A$ receptors containing the α1 subunit (McKernan et al., 2000). Further, synaptically located $GABA_A$ receptors have a α1β3γ2 subunit composition, whereas extrasynaptically located $GABA_A$ receptors have the composition α4β3δ (Rudolph et al., 2001; Ebert et al., 2002; Brown et al., 2002). In vitro electrophysiological evidence exists to suggest that whereas gaboxadol acts as a weak partial agonist at synaptically located $GABA_A$ receptors, it acts as a potent agonist at extrasynaptically located receptors (Ebert et al., 2002). Taken together, it is reasonable to suggest that the antinociceptive actions of selective $GABA_A$ receptor agonists such as muscimol and gaboxadol as shown here, may thus be mediated via extrasynaptically located receptors (Voss et al., 2003). By extension it is also reasonable to assume that α1 containing $GABA_A$ receptors may not mediate antinociception in SNI rats, based on the lack of an antinociceptive effect observed for zolpidem at a dose that produced profound motor impairment indicative of α1 receptor activation. Alternatively, attenuated GABA release within the spinal dorsal horn after nerve injury (Moore et al., 2002) may account for the lack of antinoceptive action of zolpidem, since zolpidem binding to $GABA_A$ receptors would be expected to allosterically modulate actions of endogenous GABA.

Changes in the expression of $GABA_A$ receptor channels within dorsal root ganglion neurones after nerve section imply that the periphery may be a possible site of drug action after nerve damage (Honore et al., 2000). Isoguvacine, is a potent $GABA_A$ receptor agonist and when administered intrathecally has similar effects on pain relief as muscimol (Malan et al., 2002). However, the intrinsic physio-chemical properties of isoguvacine limit its access to the central nervous system (Krogsgaard-Larsen et al., 1981). The apparent lack of antinociceptive effect obtained after systemic administration of isoguvacine in the current study suggests that antinociceptive actions of $GABA_A$ receptor agonists after nerve injury are mediated selectively within the central nervous system.

4.4. Gaboxadol and sleep

Patients who suffer from chronic pain often experience difficulties in initiating and maintaining sleep, with as many as 87% of patients having a comorbid sleep disorder more severe than in those patients where sleep disorder is the primary indication (Smith et al., 2000). Paradoxically, sleep deprivation is associated with a decreased pain threshold, muscle aches, and stiffness in normal volunteers (Nicholson and Verma, 2004). Sleep disturbances have also been reported in animal models of chronic pain. Rats with adjuvant-induced polyarthritis show marked abnormalities in sleep architecture, including increased wakefulness and a reduction in overall electroencephalogram (EEG) amplitude (Landis et al., 1989). Similarly, alterations in EEG and sleep patterns have been reported in rats with peripheral nerve injury (Andersen and Tufik, 2003; Monassi et al., 2003). Gaboxadol has been shown to improve but not induce sleep and is currently in clinical trials for treating sleep disorders (Mathias et al., 2001; Lancel et al., 2001). Taken together with the current data, these findings suggest that the administration of gaboxadol to those neuropathic pain patients with severely impacted sleep patterns, may provide a multifaceted approach to improving various signs and symptoms of neuropathic pain. Of course our study has not addressed this issue, although such animal experiments are obviously warranted.

4.5. Conclusions

The current findings suggest that subtype selective $GABA_A$ receptor agonists such as gaboxadol may have some utility in alleviating neuropathic allodynia and hyperalgesia in human patients. Furthermore, the utility of gaboxadol for improving sleep disorders may have additional benefits for neuropathic pain patients where disruption of sleep impacts negatively on quality of life measurements.

Acknowledgements

We would like to thank Poul Krogsgaard Larsen and Bente Frølund for providing gaboxadol and muscimol. Expert technical assistance was provided by Nete Ibsen and Kirtsten Metz. We would also like to thank Dr N. Mirza for critical reading of the manuscript. FR was supported by a fellowship from the Danish University of Pharmaceutical Sciences.

References

Andersen, M.L., Tufik, S., 2003. Sleep patterns over 21-day period in rats with chronic constriction of sciatic nerve. Brain Res. 984, 84–92.

Blackburn-Munro, G., 2004. Pain-like behaviours in animals—how human are they? Trends Pharmacol. Sci. 25, 299–305.

Blackburn-Munro, G., Bomholt, S.F., Erichsen, H.K., 2004. Behavioural effects of the novel AMPA/GluR5 selective receptor antagonist NS1209 after systemic administration in animal models of experimental pain. Neuropharmacology 47, 351–362.

Brown, N., Kerby, J., Bonnert, T.P., Whiting, P.J., Wafford, K.A., 2002. Pharmacological characterization of a novel cell line expressing human $\alpha4\beta3\delta$ $GABA_A$ receptors. Br. J. Pharmacol. 136, 965–974.

Bulka, A., Wiesenfeld-Hallin, Z., 2003. Comparison of response characteristics of cutaneous mechanoreceptors in normal and neuropathic Sprague–Dawley and spontaneously hypertensive rats. Neurosci. Lett. 340, 61–64.

Bulka, A., Kouya, P.F., Bottiger, Y., Svensson, J.O., Xu, X.J., Wiesenfeld-Hallin, Z., 2004. Comparison of the antinociceptive effect of morphine, methadone, buprenorphine and codeine in two substrains of Sprague–Dawley rats. Eur. J. Pharmacol. 492, 27–34.

Cui, J.G., Linderoth, B., Meyerson, B.A., 1996. Effects of spinal cord stimulation on touch-evoked allodynia involve GABAergic mechanisms. An experimental study in the mononeuropathic rat. Pain 66, 287–295.

Decosterd, I., Woolf, C.J., 2000. Spared nerve injury: an animal model of persistent peripheral neuropathic pain. Pain 87, 149–158.

Decosterd, I., Allchorne, A., Woolf, C.J., 2004. Differential analgesic sensitivity of two distinct neuropathic pain models. Anesth. Analg. 99, 457–463.

Dworkin, R.H., Backonja, M., Rowbotham, M.C., Allen, R.R., Argoff, C.R., Bennett, G.J., Bushnell, M.C., Farrar, J.T., Galer, B.S., Haythornthwaite, J.A., Hewitt, D.J., Loeser, J.D., Max, M.B., Saltarelli, M., Schmader, K.E., Stein, C., Thompson, D., Turk, D.C., Wallace, M.S., Watkins, L.R., Weinstein, S.M., 2003. Advances in neuropathic pain: diagnosis, mechanisms, and treatment recommendations. Arch. Neurol. 60, 1524–1534.

Eaton, M.J., Plunkett, J.A., Karmally, S., Martinez, M.A., Montanez, K., 1998. Changes in GAD- and GABA-immunoreactivity in the spinal dorsal horn after peripheral nerve injury and promotion of recovery by lumbar transplant of immortalized serotonergic precursors. Neuroanatomy 16, 57–72.

Ebert, B., Storustovu, S., Mortensen, M., Frolund, B., 2002. Characterization of $GABA_A$ receptor ligands in the rat cortical wedge preparation: evidence for action at extrasynaptic receptors? Br. J. Pharmacol. 137, 1–8.

Erichsen, H.K., Blackburn-Munro, G., 2002. Pharmacological characterisation of the spared nerve injury model of neuropathic pain. Pain 98, 151–161.

Frolund, B., Ebert, B., Kristiansen, U., Liljefors, T., Krogsgaard-Larsen, P., 2002. $GABA_A$ receptor ligands and their therapeutic potentials. Curr. Top. Med. Chem. 2, 817–832.

Gottrup, H., Nielsen, J., Arendt-Nielsen, L., Jensen, T.S., 1998. The relationship between sensory thresholds and mechanical hyperalgesia in nerve injury. Pain 75, 321–329.

Hao, J.-X., Xu, X.-J., Aldskogius, H., Seiger, A., Wiesenfeld-Hallin, Z., 1991. Allodynia-like effects in rat after ischaemic spinal cord injury photochemically induced by laser irradiation. Pain 45, 175–185.

Hao, J.X., Xu, I.S., Xu, X.J., Wiesenfeld-Hallin, Z., 1999. Effects of intrathecal morphine, clonidine and baclofen on allodynia after partial sciatic nerve injury in the rat. Acta Anaesthesiol. Scand. 43, 1027–1034.

Harden, N., Cohen, M., 2003. Unmet needs in the management of neuropathic pain. J. Pain Symp. Manag. 25, S12–S17.

Honore, P., Rogers, S.D., Schwei, M.J., Salak-Johnson, J.L., Luger, N.M., Sabino, M.C., Clohisy, D.R., Mantyh, P.W., 2000. Murine models of inflammatory, neuropathic and cancer pain each generates a unique set of neurochemical changes in the spinal cord and sensory neurons. Neuroscience 98, 585–598.

Jensen, T.S., Baron, R., 2003. Translation of symptoms and signs into mechanisms in neuropathic pain. Pain 102, 1–8.

Kaneko, M., Hammond, D.L., 1997. Role of spinal gamma-aminobutyric $acid_A$ receptors in formalin-induced nociception in the rat. J. Pharmacol. Exp. Ther. 282, 928–938.

Krogsgaard-Larsen, P., Schultz, B., Mikkelsen, H., Aaes-Jorgensen, T., Bogeso, K.P., 1981. THIP, isoguvacine, isoguvacine oxide, and related GABA agonists. Adv. Biochem. Psychopharmacol. 29, 69–76.

Krogsgaard-Larsen, P., Frolund, B., Kristiansen, U., Frydenvang, K., Ebert, B., 1997. $GABA_A$ and $GABA_B$ receptor agonists, partial agonists, antagonists and modulators: design and therapeutic prospects. Eur. J. Pharmacol. 5, 355–384.

Krogsgaard-Larsen, P., Frolund, B., Liljefors, T., Ebert, B., 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.

Lancel, M., Wetter, T.C., Steiger, A., Mathias, S., 2001. Effect of the $GABA_A$ agonist gaboxadol on nocturnal sleep and hormone secretion in healthy elderly subjects. Am. J. Physiol., Endocrinol. Metab. 281, E130–E137.

Landis, C.A., Levine, J.D., Robinson, C.R., 1989. Decreased slow-wave and paradoxical sleep in a rat chronic pain model. Sleep 12, 167–177.

Lu, J., Sanchez, C., Saper, C.B., Vogel, V., 2003. Gaboxadol activates endogenous sleep control mechanisms. Abstr.- Soc. Neurosci. (P617.1).

Malan, T.P., Mata, H.P., Porreca, F., 2002. Spinal $GABA_A$ and $GABA_B$ receptor pharmacology in a rat model of neuropathic pain. Anesthesiology 96, 1161–1167.

Mathias, S., Steiger, A., Lancel, M., 2001. The $GABA_A$ agonist gaboxadol improves the quality of post-nap sleep. Psychopharmacology (Berl) 157, 299–304.

McKernan, R.M., Rosahl, T.W., Reynolds, D.S., Sur, C., Wafford, K.A., Atack, J.R., Farrar, S., Myers, J., Cook, G., Ferris, P., Garrett, L., Bristow, L., Marshall, G., Macaulay, A., Brown, N., Howell, O., Moore, K.W., Carling, R.W., Street, L.J., Castro, J.L., Ragan, C.I., Dawson, G.R., Whiting, P.J., 2000. Sedative but not anxiolytic properties of benzodiazepines are mediated by the $GABA_A$ receptor α_1 subtype. Nat. Neurosci. 3, 587–592.

Mehta, A.K., Ticku, M.K., 1999. An update on $GABA_A$ receptors. Brain Res. Rev. 29, 196–217.

Monassi, C.R., Bandler, R., Keay, K.A., 2003. A subpopulation of rats show social and sleep–waking changes typical of chronic neuropathic pain following peripheral nerve injury. Eur. J. Neurosci. 17, 1907–1920.

Moore, K.A., Kohno, T., Karchewski, L.A., Scholz, J., Baba, H., Woolf, C.J., 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.

Nicholson, B., Verma, S., 2004. Comorbidities in chronic neuropathic pain. Pain Med. 5 (Suppl. 1), S9–S27.

Polgar, E., Gray, S., Riddell, J.S., Todd, A.J., 2004. Lack of evidence for significant neuronal loss in laminae I–III of the spinal dorsal horn of the rat in the chronic constriction injury model. Pain 111, 144–150.

Raja, S.N., Meyer, A.J., Ringkamp, M., Campell, J.N., 1999. Peripheral neural mechanisms of nociception. In: Wall, P.D., Melzack, R. (Eds.), Textbook of Pain. Churchill Livingstone, pp. 105–128.

Rudolph, U., Crestani, F., Mohler, H., 2001. $GABA_A$ receptor subtypes: dissecting their pharmacological functions. Trends Pharmacol. Sci. 22, 188–194.

Sieghart, W., Fuchs, K., Tretter, V., Ebert, V., Jechlinger, M., Hoger, H., Adamiker, D., 1999. Structure and subunit composition of $GABA_A$ receptors. Neurochem. Int. 34, 379–385.

Sivilotti, L., Woolf, C.J., 1994. The contribution of $GABA_A$ and glycine receptors to central sensitization: disinhibition and touch-evoked allodynia in the spinal cord. J. Neurophysiol. 72, 169–179.

Smith, M.T., Perlis, M.L., Smith, M.S., Giles, D.E., Carmody, T.P., 2000. Sleep quality and presleep arousal in chronic pain. J. Behav. Med. 23, 1–13.

Tal, M., Bennett, G.J., 1994. Extra-territorial pain in rats with a peripheral mononeuropathy: mechano-hyperalgesia and mechano-allodynia in the territory of an uninjured nerve. Pain 57, 375–382.

Voss, J., Sanchez, C., Michelsen, S., Ebert, B., 2003. Rotarod studies in the rat of the $GABA_A$ receptor agonist gaboxadol: lack of ethanol potentiation and benzodiazepine cross-tolerance. Eur. J. Pharmacol. 482, 215–222.

Woolf, C.J., Salter, M.W., 2000. Neuronal plasticity: increasing the gain in pain. Science 288, 1765–1769.

Zimmermann, M., 1983. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 16, 109–110.