The antiallodynic effect of NMDA antagonists in neuropathic pain outlasts the duration of the in vivo NMDA antagonism

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Received 30 November 2005; received in revised form 13 February 2006; accepted 15 February 2006

Abstract

Clinical reports have described a long-lasting relief in neuropathic pain patients treated with NMDA receptor antagonists; it is unclear, however, what mediates this effect. In this work, we have used two NMDA antagonists of different class to investigate if the antiallodynic effects in a rat neuropathy model can outlast their in vivo NMDA antagonism. Both the uncompetitive NMDA antagonist ketamine and the glycineB antagonist MRZ 2/576 inhibited neuronal responses to iontophoretic NMDA in anaesthetised rats with the time course consistent with their known pharmacokinetics (t1/2 ~ 10–12 min, similar in control and nerve-injured rats). Surprisingly, the antiallodynic effects of the same doses of the NMDA antagonists in the neuropathic pain model were long-lasting (>3 h with ketamine, >24 h with MRZ 2/576). The effect of ketamine was further prolonged (>24 h) when combined with a short-acting opioid, fentanyl, which only produced a short effect (~40 min) when given alone. The duration of centrally mediated side effects of ketamine and MRZ 2/576 was short, similar to the in vivo NMDA antagonism. We speculate that NMDA receptor blockade may down-regulate the central sensitisation triggered by nerve injury, resulting in a long-lasting antiallodynic effect. Development of short-acting NMDA antagonists could represent a strategy for improving their tolerability.

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Keywords: Neuropathic pain; NMDA antagonist; Opioid; Spinal cord; Alldynia; Dorsal horn neuron; Iontophoresis

1. Introduction

The important role of N-methyl-D-aspartate (NMDA) receptors in the development and maintenance of chronic pain states has been well documented (for review see Parsons, 2001; Chizh and Eide, 2002; Chizh and Headley, 2005). NMDA antagonists of different class have shown efficacy in preclinical models as well as in patients with chronic pain, including neuropathic pain. However, a large-scale clinical use of NMDA antagonists for the treatment of neuropathic pain is limited by unacceptable side effects (hallucinations, sedation, ataxia) of currently available compounds of this class (Parsons, 2001; Chizh and Eide, 2002; Chizh and Headley, 2005). Several clinical studies have observed a long-lasting relief in some neuropathic pain patients treated with NMDA antagonists (Pud et al., 1998; Eisenberg and Pud, 1998; Rabben et al., 1999; Correll et al., 2004). However, the compounds shown to have such long-lasting effects, amantadine and ketamine, are both channel blockers, the former being a relatively weak and non-selective NMDA antagonist (Parsons et al., 2000), and the latter having a variety of activities other than the NMDA receptor antagonism (Eide et al., 1997; Chizh and Eide, 2002). It remains, therefore, unclear, whether the observed “curative” antiallodynic action of these compounds is related to the NMDA receptor antagonism, and whether this is a common feature of NMDA antagonists of different class. Specifically, NMDA antagonists acting at the glycineB site
of the receptor do not produce the psychotomimetic and neurotoxic effects typical for uncompetitive antagonists such as phencyclidine, dizocilpine (MK-801) and ketamine (Danysz et al., 1998; Danysz and Parsons, 1998). Some compounds of this class have been reported to be selective and short-acting NMDA antagonists in vivo (Parsons et al., 1997).

In the present work, we have investigated the hypothesis that a short blockade of NMDA receptors may lead to a longer-lasting alleviation of neuropathic pain. We tested the anti-allodynic effects of two short-acting NMDA antagonists of different class, namely, the NMDA receptor channel blocker, ketamine, and the glycineB antagonist, MRZ 2/576, in a rat model of neuropathic pain, and compared their potency and time course with those of the antagonism of NMDA receptors on dorsal horn neurones in the rat spinal cord in vivo. We also tested if the antiallodynic effect of ketamine can be further prolonged by a short-acting opioid, fentanyl. The rationale for this experiment is based on the evidence that opioids can inhibit glutamate release (Malmberg and Yaksh, 1995; Ueda et al., 1995), and hence may act synergistically with NMDA antagonists in blocking NMDA receptor-mediated hyperexcitability. Some of this work has previously been reported in abstract form (Chizh et al., 2001).

2. Materials and methods

Adult male Sprague–Dawley rats (170–310 g, obtained from Iffa Credo, Belgium, or Janvier, France) were used in the study. Animals were kept under standard laboratory conditions with free access to standard laboratory food and tap water. The experiments were performed in accordance with German and ECC guidelines (86/609/EEC) for the use of laboratory animals. All efforts were made to minimise animal suffering and to reduce the number of animals used. Although the operators performing behavioural tests were not formally “blinded” with respect to the treatment, they were not aware of the nature of the differences between the drugs and the study hypothesis.

2.1. Cold allodynia in rats with chronic constriction injury (CCI)

The rat chronic constriction injury model was utilised according to Bennett and Xie (1988). Animals were anaesthetised with pentobarbital (Nembutal, 50 mg/kg, i.p.), and the right common sciatic nerve was exposed by blunt dissection at the mid-thigh level. Four loose ligatures (5-0 chromic catgut) were placed around the nerve taking care not to interrupt the epineural circulation. After the operation, the animals were allowed to recover for one week. During this period they typically developed cold allodynia, which was stable for at least 5 weeks. Cold allodynia was tested on a metal plate cooled by a water bath to a constant temperature of 4 °C. Whereas intact rats did not show any brisk paw withdrawals on the cold plate at all, CCI rats typically showed 10–15 reactions per minute. Hence, these characteristic responses of nerve-injured rats were counted as a measure of allodynia. Animals, randomly assigned to groups of eight for each test dose and vehicle, were observed for periods of 2 min before and after the application of test compound and the number of brisk withdrawal reactions was counted. Several test points after application were tested. Percentages of the maximal possible effect (MPE) for each time point were determined with the pre-drug value taken as 0% MPE and total lack of withdrawal reactions during the testing period as 100% MPE. The data are presented as mean ± S.E.M. Significance was calculated by two-way ANOVA with one factor repeated measures and least squares means post hoc analysis versus the vehicle group on the percent MPE values; P < 0.05 was deemed significant.

2.2. NMDA-evoked activity of dorsal horn neurones

The NMDA-antagonistic activity and selectivity of the test compounds in the spinal cord were examined on responses of spinal dorsal horn neurones to iontophoretically applied NMDA and (RS)-2-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA). The experimental details can be found elsewhere (see Chizh et al., 1997). In brief, tracheal, carotid and jugular cannulations and a laminectomy (T10–L2) were performed under halothane anaesthesia. The spinal cord was not cut. The animal was placed in a recording frame, and anaesthesia switched to α-chloralose (50 mg/kg i.v. initially, then i.v. infusion at 10–20 mg/kg per hour). Blood pressure was monitored continuously. The adequacy of anaesthesia was verified by the stability of cardiovascular parameters and lack of blood pressure responses to noxious stimuli. Core temperature was maintained at 37 ± 0.5 °C. Multi-barrel glass electrodes were used for extracellular recordings of single unit activity of dorsal horn wide dynamic range (WDR) neurones (via the central barrel, filled with 3.5 M NaCl, typical resistance 2.5–4.5 MΩ) and for iontophoretic applications of NMDA (100 nM) in 100 mM NaCl) and AMPA (10 mM in 200 mM NaCl; typical resistances 25–45 MΩ for both). Automated current balancing via one of the outer barrels (filled with 150 mM NaCl; 20–50 MΩ resistance) was employed routinely. Single unit activity of spinal dorsal horn neurones was monitored on a digital oscilloscope. Amino acids were ejected as anions in computer-controlled cycles (40 s ejection, 30 s interval between NMDA and AMPA applications, total cycle length 200 s); a retaining voltage of ±500 mV was applied during the intervals to prevent leakage of the amino acids. Counts of amino-acid evoked spikes were analysed in stimulus-related epochs. Drugs were injected i.v. after at least three stable response cycles showing <10% variability. Drug effects were expressed as percentages of control, which was the average of the last three pre-drug values. Statistical analysis was carried out by paired Student’s t-test; the level of significance was set at P < 0.05. The data are presented as mean ± S.E.M. ED50 calculations were done by sigmoid curve fitting using Fig.P software (Biosoft, UK). When less than three doses were tested then approximate ED50 values were calculated. With ketamine, electrophysiological tests were carried out in normal animals and in those subjected to CCI. In the latter case, the presence of allodynia was confirmed in all animals prior to electrophysiological testing. The electrode was inserted ipsilaterally to the site of CCI.

2.3. Open-field motility test in rats

The possible sedative and muscle relaxant actions of the compounds were evaluated in the open field test (MotiSystem, TSE, Bad Homburg, Germany). In this system, 32 infrared emitters and opposed sensors are evenly distributed on the length of the X- and Y-axis of the test field (45 × 45 cm). After the administration of the test compound, rats were placed individually into the centre of the open field and the activity was measured for 5 min. Locomotor activity was quantified as the number of light beam interruptions, and the overall movement distance (metres) over this period was calculated. Control groups received vehicle injection. Drug effects were normalised with respect to the control group data.

2.4. Drug administration

Drugs tested in this study were racemic ketamine (Ketavet, Parke-Davis, UK), fentanyl (Janssen-Cilag, Germany) and MRZ 2/576 (Merz Pharmaceuticals, Germany). They were dissolved in 0.9% saline (ketamine, fentanyl) or 5.5% 5-fructose (MRZ 2/576) and administered i.v. (1 ml/kg) or i.p. (3 ml/kg). NMAD and AMPA for iontophoretic tests were from Tocris Cookson (U.K.).

3. Results

The competitive NMDA antagonist ketamine selectively blocked NMDA receptor-mediated responses of spinal WDR neurones in the spinal cord of normal animals (Fig. 1). The duration of the NMDA antagonism by ketamine was short (half-recovery time of approximately 10–12 min, see Fig. 1A) and
consistent with the known pharmacokinetics of the drug (Cohen et al., 1973; Sweetman, 2005). In all cases, a full recovery was observed in 30−40 min after the drug administration. The potency of the NMDA antagonism by ketamine (ED50 of approximately 2 mg/kg, see Fig. 2) was similar to that in the previous report (Parsons et al., 1997). The time course of the NMDA antagonism by MRZ 2/576 was similar to that of ketamine (half-recovery of approximately 10−12 min, see Fig. 2); a full recovery of NMDA-evoked activity of spinal WDR neurones was observed in 30−40 min.

In behavioural experiments in CCI rats, ketamine (4.64 mg/kg, i.v.) produced a significant antiallodynic effects that lasted for several hours (>3 h, P = 0.0007 at 180 min, see Fig. 3), much longer than the duration of NMDA antagonism in the spinal cord. In contrast, the short-acting μ-opioid agonist fentanyl (0.01 mg/kg, i.v.) caused a significant effect in this model with a time course consistent with its known pharmacokinetics (Cohen et al., 1973); a near-complete recovery from this effect was observed in approximately 1 h after administration (P = 0.2599 at 60 min, see Fig. 3). When fentanyl and ketamine were co-administered in the same animals, the magnitude and duration of the significant antiallodynic effect was greater than with either of the drugs alone; a small but significant antiallodynic effect was still observed 24 h after their administration (P = 0.0074 at 24 h, see Fig. 3). Statistical evaluation of the four treatment groups revealed significant time, treatment and interaction effects (time F(7,168) = 16.28, P < 0.0001; ketamine: treatment F(1,24) = 78.40, P < 0.0001, interaction F(7,168) = 9.70, P < 0.0001; fentanyl: treatment F(1,24) = 50.30, P < 0.0001, interaction F(7,168) = 16.33, P < 0.0001; combination: treatment F(1,24) = 0.28, P = 0.6044, interaction F(7,168) = 2.44, P = 0.0207). The post hoc analysis revealed that antiallodynic effect of the combination at 24 h was significantly different not only from the vehicle group (P = 0.0074), but also from the respective values in the ketamine (P = 0.0080) and the fentanyl (P = 0.0073) groups. Importantly, ketamine-induced side effects (ataxia, sedation, hyperactivity) were only observed during the
Fig. 3. The time course of the effects of ketamine (4.64 mg/kg, i.v., n = 7), fentanyl (0.01 mg/kg, i.v., n = 7) and their combination (n = 8) on cold allodynia in animals with sciatic nerve injury. Note that although the effect of fentanyl on cold allodynia was short (∼1 h), the drug prolonged the duration of the antiallodynic effect of ketamine (*significantly different from the control group (n = 6), P < 0.05, least squares means post hoc analysis). Side effects (ataxia, sedation or hyperactivity) in the ketamine and ketamine + fentanyl group were observed only during the first 15–30 min after the drug. During the 3-h observation period, the effects in the ketamine and ketamine + fentanyl group were significantly different from the vehicle group (*P < 0.05, least squares means post hoc analysis on percentage MPE values).

Fig. 4. The time course of the effect of MRZ 2/576 (2.15–10 mg/kg, i.p., each group n = 8) on cold allodynia in animals with sciatic nerve injury. (A) The 24 h time course of the antiallodynic effect of the three doses of MRZ 2/576 vs. vehicle. (B) Comparison between 3 h, 24 h and 8 days post-dose for the three doses of MRZ 2/576. Side effects (ataxia, sedation) were observed with all three doses only during the first 15–30 min after the drug. With all three doses of MRZ 2/576, the effects were significantly different from the vehicle group during the first 24 h post dose (*P < 0.05, least squares means post hoc analysis on percentage MPE values), but not 8 days post dose.

first 15–30 min after the drug administration, consistent with the time course of the NMDA antagonism by the drug.

Similar to the uncompetitive NMDA antagonist ketamine, the glycineB antagonist MRZ 2/576 (2.15–10 mg/kg, i.p.) significantly (treatment F(3,27) = 15.88, P < 0.0001; time F(7,189) = 27.68, P < 0.0001; treatment × time F(21,189) = 5.15, P < 0.0001) alleviated cold allodynia in CCI animals; the duration of the antiallodynic effect substantially outlasted the time course of NMDA antagonism in electrophysiological tests (see Fig. 4). Although the effect appeared dose-dependent in the first 15–30 min after administration, there was no clear dose-dependency at later time-points. When tested 24 h after the compound administration, the effect was still present and significant (P < 0.0001, P = 0.0006, P = 0.0001 for 2.15, 4.64 and 10 mg/kg at 24 h, respectively, Fig. 4). Interestingly, the magnitude of the antiallodynic effect with all three doses of MRZ 2/576 was similar between the 3 h and 24 h time-points; however, a complete return to baseline levels of allodynia was observed 8 days after the dosing (P > 0.05, Fig. 4). As with ketamine, the duration of centrally mediated side effects (ataxia, sedation) was rather short and consistent with the time course of the NMDA antagonism (15–30 min). In the open field test, the i.p. administration of MRZ 2/576 (15 min prior to testing) showed a dose dependent reduction in locomotor activity, with an ED50 of 5.21 mg/kg (confidence interval 4.42–6.23 mg/kg).

4. Discussion

The main finding of our work is that the antiallodynic effect of NMDA antagonists of different class substantially outlasts the duration of NMDA receptor antagonism at the presumed site of action, i.e. in the spinal cord. As indicated by electrophysiological experiments, the time course of NMDA antagonism in the spinal cord for both the uncompetitive antagonist ketamine and the glycineB antagonist MRZ 2/576 is consistent with their pharmacokinetics. Thus, the distribution half-life time of ketamine is approximately 10–15 min (Cohen et al., 1973; Sweetman, 2005). Likewise, the half-life time of MRZ 2/576 in the rat is 15.6 ± 1.3 min in the serum and 20.2 ± 0.2 min in the brain (as measured by microdialysis, Hesselink et al., 1999). With both compounds, the duration of centrally mediated side effects is consistent with their pharmacokinetics and similar to that of the NMDA antagonism in the spinal cord. This suggests that a short (approximately 30 min) block of NMDA receptors does not cause any long-lasting changes in physiological transmission. Furthermore, the time course and potency of NMDA antagonism by the uncompetitive NMDA antagonist ketamine are similar in normal rats and animals with chronic constriction injury to the sciatic nerve, indicating that the central sensitisation underlying neuropathic pain does not change the kinetics of NMDA antagonist interaction with the receptor.

The dissociation between the pharmacokinetics and pharmacodynamic effects becomes apparent in the model of neuropathic pain. Consistent with the vast literature on NMDA
antagonists in neuropathic pain (reviewed by Fisher et al., 2000; Sang, 2000; Parsons, 2001; Chizh and Eide, 2002; Chizh and Headley, 2005), both ketamine and MRZ 2/576 showed profound antiallodynic effects in the present study. However, with both of these short-acting NMDA antagonists, the duration of these effects is substantially longer than the duration of the NMDA antagonism in the spinal cord of rats. Although this is the first study directly demonstrating such dissociation, our findings are broadly in accord with several preclinical and clinical observations. Preclinically, i.t. ketamine given preemptively was shown to cause a long-lasting delay in the development of alldynia associated with spinal nerve injury in rats (Burton et al., 1999). Several experimental studies in human volunteer models have demonstrated that low doses of ketamine can inhibit established central sensitisation (Park et al., 1995; Warncke et al., 1997; Gottrup et al., 2000; Koppert et al., 2001; Wang et al., 2005; cf. Wallace et al., 2002), whereby in at least one study the duration of this inhibition appeared to be substantially longer than the i.v. infusion (Koppert et al., 2001). In the clinic, perioperative administration of NMDA antagonists (pre-emptive analgesia) has been shown by several groups to suppress the development of post-operative pain (Tverskoy et al., 1994; Fu et al., 1997; cf. Mathisen et al., 1999; Aida et al., 2000; Jaksch et al., 2002). Finally, several patient studies have reported that the duration of relief of neuropathic pain by NMDA antagonists may substantially outlast their known pharmacokinetic time course (Pud et al., 1998; Eisenberg and Pud, 1998; Rabben et al., 1999). A single injection or infusion of a low dose of ketamine has been reported to produce pain relief lasting up to 24 h in some patients with trigeminal neuropathy (Rabben et al., 1999), or even as long as 6 months in some patients with complex regional pain syndrome (Correll et al., 2004; cf. Max et al., 1995). With another uncompetitive NMDA antagonist, amantadine, pain relief has been reported to last 5–7 months after a short-term treatment (Eisenberg and Pud, 1998). It should be borne in mind, however, that amantadine is a very weak NMDA antagonist, and the contribution of this mechanism to its overall efficacy is not clear.

The mechanisms underlying the long duration of antiallodynic effects with NMDA antagonists are not clear. In addition to their role in the induction of neural hyperexcitability (by mediating pro-nociceptive plasticity such as wind-up), NMDA receptors are involved in the maintenance of central sensitisation. In various pain states associated with peripheral nerve pathology, central sensitisation appears to be maintained by ongoing barrage from the periphery (e.g. Fields et al., 1998; Petersen et al., 2000). In these situations, NMDA antagonists may exert their action by reducing the central sensitisation both directly and indirectly (i.e. by prevention of re-sensitisation by peripheral input). In central pain states, where sensitisation appears to be independent from peripheral input, NMDA antagonists have also shown efficacy, presumably by a direct effect on established central sensitisation (e.g. Eide et al., 1995). Our results supported by clinical observations imply that NMDA receptor blockade can lead to long-term re-setting (down-regulation) of the level of excitability. Interestingly, similar findings have been made with short-acting NMDA antagonists reversing tolerance to opioids, a condition associated with the development of NMDA receptor-mediated hyperalgesia (Belotserzeva et al., 2000; Danyusz et al., 2005). It is tempting to speculate that pro-nociceptive molecular mechanisms downstream of the NMDA receptor may be switched off by a relatively short desensitisation in the pain pathway. These interactions may be specific for the pro-nociceptive sensitisation as the duration of the anticonvulsant effect of short-acting glycine\(_\beta\) NMDA antagonists appeared to be in line with their pharmacokinetics (Parsons et al., 1997; Hesselink et al., 1999).

It should be noted that there is a possibility that the long antiallodynic effects observed in our study are not mediated via NMDA receptors. However, as discussed above, the pharmacokinetic lives of both ketamine and Mrz 2/576 (in plasma and brain) are short and consistent with the duration of inhibition of NMDA responses and side effects. Hence the only possibility of an “off-target” activity would be via their metabolites. Although ketamine is extensively metabolised in the liver, Mrz 2/576 is predominantly eliminated via renal excretion (Hesselink et al., 1999); hence this mechanism appears very unlikely.

Another potentially important finding of this work is the interaction of two short-acting analgesics with different mechanisms leading to a prolonged antiallodynic effect in neuropathic pain. The \(\mu\)-opioid fentanyl only caused a relatively short reversal of alldynia when given alone, but substantially prolonged the antiallodynic action of ketamine. Opioids have been shown to inhibit glutamate release in the spinal cord evoked by noxious stimuli (Malmberg and Yaksh, 1995; Ueda et al., 1995) and hence could act synergistically with NMDA antagonists at blocking NMDA-receptor mediated plasticity. One can speculate that a short \(\mu\)-opioid receptor-mediated inhibition of glutamate release in the pathway where nerve injury has resulted in NMDA receptor-mediated sensitisation may not be sufficient to cause a long-lasting inhibition alone, but could potentiate the effect of NMDA receptor blockade. This suggests a potentially useful combination strategy for the treatment of neuropathic pain.

In conclusion, our findings imply that a short block of NMDA receptors in the spinal cord can lead to a long-lasting down-regulation of the central hyperexcitability triggered by peripheral nerve injury, the effect being a common feature of different classes of NMDA antagonist. The use of short-acting NMDA antagonists, therefore, offers a potentially useful strategy for separating the analgesic effect of NMDA antagonists in neuropathic pain from side effects.

Acknowledgements

This work was supported by Grünenthal GmbH. We are grateful to Mr B. Liebenhoff, Mr H. Schlütz, Mrs E. Schumacher and Mr T. Vanderbrück (all of Grünenthal GmbH R&D) for excellent technical assistance.

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