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Pharmacological characterization of the chronic constriction injury model of neuropathic pain

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Abstract

The chronic constriction injury model is a rat model of neuropathic pain based on a unilateral loose ligation of the sciatic nerve. The aim of the present study was to test its sensitivity to various clinically validated and experimental drugs. Mechanical allodynia and thermal hyperalgesia developed within one week post-surgery and were reliably present for at least 7 weeks. Mechanical allodynia was strongly attenuated by morphine (minimal effective dose in brackets: 8 mg/kg, p.o.) and the cannabinoids Δ^9-tetrahydrocannabinol (3 mg/kg, p.o.) and (−)-*cis*-3-[2-hydroxy-4(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl) cyclohexanol (CP 55,940; 0.05 mg/kg, i.p.), and weakly/moderately attenuated by the anticonvulsants gabapentin (50 mg/kg, i.p.) and carbamazepine (32 mg/kg, i.p.), the muscle relaxant baclofen (3 mg/kg, i.p.), and the adenosine kinase inhibitor 4-amino-5-(3-bromophenyl)-7-(6-morpholino-pyridin-3-yl)pyrido[2,3-*d*]pyrimidine (ABT-702; 30 mg/kg, i.p.). Thermal hyperalgesia was strongly attenuated by morphine (16 mg/kg, p.o.), Δ^9-tetrahydrocannabinol (6 mg/kg, p.o.), CP 55,940 (0.025 mg/kg, i.p.), carbamazepine (32 mg/kg, i.p.) and the antidepressant amitriptyline (32 mg/kg, p.o.), and weakly/moderately attenuated by gabapentin (50 mg/kg, i.p.), the anti-inflammatory cyclooxygenase-2 inhibitor rofecoxib (30 mg/kg, i.p.) and the adenosine A_1 receptor positive allosteric modulator 2-amino-4,5,6,7-tetrahydrobenzo(*b*)thiophen-3-yl 4-chlorophenylmethanone (T62; 30 mg/kg, i.p.). Both symptoms were hardly or not affected by the nonselective *N*-methyl-D-aspartate receptor antagonists ketamine and dizocilpine, and the *N*-methyl-D-aspartate receptor NR2B-selective antagonists ifenprodil and *R*-(*R**,*S**)-α-(4-hydroxyphenyl)-β-methyl-4-(phenyl-methyl)-1-piperidine propranol (Ro 25-6981). The finding that mechanical allodynia and/or thermal hyperalgesia are attenuated by various established compounds further supports the validity of the chronic constriction injury model for the study of neuropathic pain and its use for the identification of novel treatments.

Keywords: Analgesics; Animal model; Chronic pain; Nociception; (Rat)

1. Introduction

Chronic neuropathic pain resulting from peripheral nerve injury is characterized by pathological symptoms, such as hyperalgesia and allodynia to mechanical and thermal (heat or cold) stimuli, as well as spontaneous pain, and is generally considered to respond relatively poorly to pharmacotherapy (Koltzenburg, 1998; Sindrup and Jensen, 1999). In order to study the neurobiological mechanisms underlying the development of neuropathic pain and to find novel and potentially more effective treatments, a number of rat models have been developed during the last decade (Martin and Eisenach, 2001). These models are based on either a unilateral ligation of the sciatic or spinal nerves (i.e., the chronic constriction injury model, Bennett and Xie, 1988; the partial sciatic nerve injury model, Seltzer et al., 1990; and the spinal nerve ligation model, Kim and Chung, 1992), or on a unilateral transection of one or more of the three distal branches of the sciatic nerve (i.e., the spared nerve injury model, Decosterd and Woolf, 2000; and the tibial nerve injury model, Hofmann et al., 2003).

The chronic constriction injury model appears to be one of the most frequently used models for the study of neuropathic pain and its treatment. The model, which is based on a unilateral loose ligation of the sciatic nerve, shows many of the pathophysiological properties of chronic neuropathic pain in human subjects (Bennett and Xie, 1988). It also shows a relatively high degree of similarity with other models of neuropathic pain in terms of the time-

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course and the degree of allodynia and hyperalgesia against mechanical and/or thermal stimuli, the occurrence of spontaneous pain, and its sensitivity to sympathectomy (for discussion and references, see Bridges et al., 2001; Hofmann et al., 2003). In addition, the chronic constriction injury model has been demonstrated to be sensitive to a number of compounds which are used clinically for the symptomatic treatment of chronic neuropathic pain (for review of clinical efficacy, see Sindrup and Jensen, 1999). Thus, it was reported that hyperalgesic and/or allodynic reactions to thermal and/or mechanical stimuli could be attenuated by the opiate morphine (Attal et al., 1991; Koch et al., 1996; Pelissier et al., 2003), the tricyclic antidepressant amitriptyline (Koch et al., 1996), the anticonvulsants gabapentin (Boyce et al., 1999; Hunter et al., 1997; Xiao and Bennett, 1996) and carbamazepine (Koch et al., 1996), the muscle relaxant baclofen (Smith et al., 1994), and the dissociative anaesthetic ketamine (Pelissier et al., 2003). It should be noted, however, that these reference compounds were mostly studied singularly (i.e., in separate studies), and that their efficacy was assessed under different experimental conditions and against different modalities using different behavioral readouts. Due to this lack of standardization, the relative potency and efficacy of these compounds remains unclear, making it more difficult to appraise the potential of novel experimental compounds. Recently, the pharmacological sensitivity to a number of reference compounds as assessed under standardized conditions has been reported for the spared nerve injury model (Erichsen and Blackburn-Munro, 2002) and the tibial nerve injury model (Hofmann et al., 2003).

The aim of the present study was to assess the pharmacological sensitivity of the chronic constriction injury model to the reference compounds mentioned above, as well as the novel anti-inflammatory cyclooxygenase-2 inhibitor rofecoxib (Chan et al., 1999), and to a number of experimental compounds which were reported to have antihyperalgesic and/or anti-allodynic properties in this model or in other model(s) of neuropathic pain. The experimental compounds included the cannabinoids Δ^9-tetrahydrocannabinol (Gaoini and Mechoulam, 1964) and (−)-*cis*-3-[2-hydroxy-4(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl) cyclohexanol (CP 55,940, Johnson and Melvin, 1986), the adenosine kinase inhibitor 4-amino-5-(3-bromophenyl)-7-(6-morpholino-pyridin-3-yl)pyrido[2,3-*d*]pyrimidine (ABT-702, Kowaluk et al., 2000) and the adenosine A_1 receptor positive allosteric modulator 2-amino-4,5,6,7-tetrahydrobenzo(*b*)thiophen-3-yl 4-chlorophenylmethanone (T62; Li et al., 2002), the nonselective *N*-methyl-D-aspartate receptor antagonist dizocilpine (MK-801, Wong et al., 1986), and the *N*-methyl-D-aspartate receptor NR2B-selective antagonists ifenprodil (Avenet et al., 1997) and *R*-(*R**, *S**)-α-(4-hydroxyphenyl)-β-methyl-4-(phenyl-methyl)-1-piperidine propranol (Ro 25-6981, Mutel et al., 1998). Efficacy of the compounds against thermal hyperalgesia and mechanical allodynia was assessed after acute administration. In order to estimate the specificity of the antihyperalgesic and anti-allodynic effect, behavioral reactivity was also tested at the contralateral non-operated paw. An antihyperalgesic or anti-allodynic effect was considered to be specific if it could be demonstrated that it occurred at a dose which did not affect reactivity of the non-operated paw.

2. Materials and methods

2.1. Animals and housing conditions

Male Wistar rats (180–200 g; strain HsdCpb:WU) were housed in groups of two in Makrolon® type 3 cages (22 × 37 cm, height 15 cm) under standardized conditions. Room temperature and relative humidity were maintained at 22 ± 1 °C and 55 ± 5%, respectively, and lights were on from 7:00 a.m. to 7:00 p.m. Conventional rat chow (Type 1324, Altromin, Lage, Germany) and tap water were given ad libitum. Animals could adapt to the laboratory conditions for at least 1 week before surgery took place. Experimental protocols and conditions conformed to the local regulations on animal welfare and to the Ethical Guidelines of the International Association for the Study of Pain (Zimmermann, 1983).

2.2. Surgery

The method described by Bennett and Xie (1988) was generally followed. Rats were anaesthetized with pentobarbital (Nembutal, Sanofi, Libourne, France; 50 mg/kg, i.p.). The right common sciatic nerve was exposed at the level of the middle of the thigh by blunt dissection through the biceps femoris. Proximal to the sciatic's trifurcation, about 12 mm of nerve was freed of adhering tissue and four ligatures (100% mercerized cotton thread; strength: 40) were tied loosely around it with about 1 mm spacings. The length of nerve thus affected was 6–8 mm long. Great care was taken to tie the ligatures such that the diameter of the nerve was seen to be just barely constricted when viewed with 40× magnification. The desired degree of constriction retarded, but did not arrest, circulation through the superficial epineural vasculature and sometimes produced a small, brief twitch in the muscle surrounding the exposure. The incision was closed in layers. In sham-operated controls, an identical operation was performed on the same side, except that the sciatic nerve was not ligated.

2.3. Assessment of mechanical allodynia and thermal hyperalgesia

Mechanical allodynia and thermal hyperalgesia were assessed bilaterally as described by Hofmann et al. (2003). Prior to behavioral testing, the animals were accustomed to the test cages for 5 min. Mechanical allodynia was measured by means of a pressure transducer (electronic von Frey

Anaesthesiometer, IITC-Life Science Instruments, Woodland Hills, CA). Animals were placed in a test cage with a wire mesh floor, and the tip of the anaesthesiometer was applied to the middle of the plantar surface of the operated and the non-operated hindpaw. Withdrawal threshold was measured once per trial and expressed as tolerance level in grams. Thermal sensitivity of the hindpaws was measured using the plantar test (Ugo Basile, Comerio, Italy) as described by Hargreaves et al. (1988). Animals were placed in plastic cages on the surface of a glass plate. The heat stimulus was applied from beneath, to the middle of the plantar surface by means of a radiant heat source. Thermal sensitivity was recorded once per trial as withdrawal latency in seconds with a cutoff time of 32 s.

Before the start of the pharmacological experiments, mechanical and thermal sensitivity was assessed in a group of ligated ($n=18$) and sham-operated ($n=7$) rats, tested repeatedly at different time points following surgery (i.e., days 8–12, 13–17, 20–22, 25–29, 30–34 and 45–50 post-surgery). In order to avoid additional stress shortly after the surgical procedures, pain tests were not performed during the initial week post-surgery.

2.4. Pharmacological testing

Pharmacological testing was performed between week 1 and week 5 post-surgery and generally occurred in different collectives of ligated groups (and their sham-operated controls). Baseline thermal hyperalgesia and mechanical allodynia was checked on the day before pharmacological testing, in order to ascertain behavioral pathology. Baseline values were considered to be valid if the plantar test values were around 20 and 10 s in the sham and ligated groups, respectively, and the von Frey test values were around 70 and 35 g, respectively. Animals which reliably showed both symptoms were selected for pharmacological testing on the next day. Each group included for pharmacological testing consisted of 9–14 rats (receiving either vehicle, or one of two to three doses of a compound).

The effect of acute administration of morphine (8, 16 and 32 mg/kg, p.o. $t=60$ min; one experiment took place 6–9 days post-surgery; a replication was performed in a separate group of rats, 13–17 days post-surgery), amitriptyline (32, 64 and 128 mg/kg, p.o., $t=60$ min; 6–12 days post-surgery), baclofen (0.3, 1 and 3 mg/kg, i.p., $t=30$ min; 20–23 post-surgery), gabapentin (50 and 100 mg/kg, i.p., $t=60$ min; 27–31 days post-surgery), carbamazepine (16, 32 and 64 mg/kg, i.p., $t=30$ min; 19–23 days post surgery), Δ^9 tetrahydrocannabinol (3 and 6 mg/kg, p.o., $t=60$ min; each dose tested in a separate experiment performed 26–30 and 32–37 days post-surgery, respectively), CP 55,940 (0.025, 0.05 and 0.1 mg/kg, i.p., $t=30$ min; 9–17 days post-surgery), rofecoxib (10 and 30 mg/kg, p.o., $t=60$ min; 26–30 days post surgery), ABT 702 (3, 10 and 30 mg/kg, i.p., $t=30$ min; 5–9 days post-surgery), T62 (15 and 30 mg/kg, i.p., $t=90$ min; 18–23 days post surgery), ketamine (5, 10 and 20 mg/kg, i.p., $t=30$ min; 5–10 days post surgery), dizocilpine (0.125, 0.25 and 0.5 mg/kg, i.p., $t=30$ min; 13–17 days post surgery), ifenprodil (5, 10 and 20 mg/kg, i.p., $t=30$ min; 25–30 days post surgery), and Ro 25 6981 (5, 10 and 20 mg/kg, i.p., $t=30$ min; 13–16 days post-surgery) was tested after ligation, and compared with the effect of administration of the respective vehicle in an accompanying group of ligated- and sham-operated control rats.

2.5. Drugs

Morphine HCl (Merck, Darmstadt, Germany), amitriptyline (RBI, Natick, MA), (−)-Δ^9-tetrahydrocannabinol (Sigma-Aldrich Chemie, Steinheim, Germany), and rofecoxib [MK-0966; 4-(4′-methylsulfonylphenyl)-3-phenyl-2-(5*H*)-furanone; extracted from Vioxx®, Merck, by the Chemistry Department of Bayer HealthCare, Wuppertal, Germany] were administered p.o.; whereas baclofen, carbamazepine, Ro 25-6981 [*R*-(*R**,*S**)-α-(4-hydroxyphenyl)-β-methyl-4-(phenyl-methyl)-1-piperidine propranol, (+)-Ketamine; all from Sigma-Aldrich], dizocilpine [(+)-MK-801; (+)-5-methyl-10,11-dihydroxy-5*H*-di-benzo(*a*,*d*)-cyclohepten-5,10-imine; RBI); ifenprodil hemitartrate (Tocris, Bristol, UK); gabapentin (extracted from Neurontin®, Parke-Davis, by the Chemistry Department of Bayer HealthCare), T62 (2-amino-4,5,6,7-tetrahydrobenzo(*b*)thiophen-3-yl 4-chlorophenylmethanone), ABT-702 (4-amino-5-(3-bromophenyl)-7-(6-morpholino-pyridin-3-yl)pyrido[2,3-*d*]pyrimidine) and CP 55,940 [(−)-*cis*-3-[2-hydroxy-4(1,1-dimethylheptyl)-phenyl]-*trans*-4-(3-hydroxypropyl) cyclo-hexanol; all synthesized by the Chemistry Department of Bayer HealthCare] were administered i.p. Ketamine and dizocilpine were dissolved in saline (0.9% NaCl). Morphine, amitriptyline and Δ^9-tetrahydrocannabinol were administered in 10% cremophor (Cremophor EL®, Fluka Chemie, Buchs, Switzerland) and distilled water; baclofen in a mixture of 5% DMSO (dimethylsulfoxide; Merck), 2% ethanol (ethanol absolute, 99.8%; Riedel-de Haën, Seelze, Germany) and saline; gabapentin, carbamazepine, CP 55,940 and ifenprodil in 2.5–5% Solutol® HS 15 (12-hydroxystearic-acid ethoxylate; BASF, Ludwigshafen, Germany), 2.5–5% ethanol and saline or distilled water; rofecoxib and Ro 25-6981 in 0.5% carboxymethylcellulose (Fluka Chemie) and distilled water or saline; ABT-702 in a mixture of 10% DMSO and 34% 2-hydroxypropyl β-cyclodextrin (Fluka Chemie) and saline; and T62 in 45% 2-hydroxypropyl β-cyclodextrin and saline. Compounds were administered in an application volume of 5 ml/kg body weight (except for ABT-702, which was given in an application volume of 3 ml/kg).

2.6. Statistical analysis

Data were analyzed, for each hindpaw separately, by one-way analysis of variance (ANOVA), followed, where appropriate, by Tukey's post hoc comparisons. In the case of morphine and Δ^9-tetrahydrocannabinol, data obtained from

the different experiments were pooled for data analysis and graphical presentation. The lowest dose which resulted in a statistically significant ($P<0.05$) effect, as compared with vehicle treatment, was considered to be the minimal effective dose (MED). Efficacy was calculated as the difference of the mean values obtained in the drug- and the vehicle-treated ligated groups, expressed as a percentage of the difference of the mean values obtained in the vehicle-treated sham-operated group and the vehicle-treated ligated group. Whenever efficacy level surpassed 50%, least-square linear regression analysis was used to estimate effective $dose_{50}$ (ED_{50}) values and the corresponding 95% confidence limits after log-probit conversion of the data.

3. Results

3.1. General behavior

After ligation, occasional uplifting of the affected paw was observed and all animals tended to avoid to stand on it. Uplifting of the rats resulted in stretching of the toes of the unaffected limb (as observed in naive rats); whereas the affected paw remained closed. General behavior and social interactions were not obviously changed compared with naive and sham-operated rats, and autotomy of the operated limb was very rare (<2% of all ligated rats; these animals were discarded from the experiments). Body weight developed normally and identically in all groups.

3.2. Thermal hyperalgesia and mechanical allodynia: time dependency

Thermal hyperalgesia and mechanical allodynia developed within one week following surgery, and lasted for at least 7 weeks (Fig. 1; ANOVA of the reaction latency and withdrawal threshold data obtained at the paw of the right, operated hindlimb indicated a main treatment effect; $F(1,23)=187.70$, $P<0.001$ and $F(1,23)=829.69$, $P<0.001$, respectively). Mean (S.E.M.) reaction latencies obtained at the left, non-operated, paw ranged from 20.0 (1.2) to 25.0 (1.4) across the different time points in the ligated group, and were not significantly different from the latency values obtained in the sham-operated group, which ranged from 17.1 (1.3) to 26.4 (2.7) ($F(1,23)=0.47$, $P>0.05$). Withdrawal threshold values obtained at the left paw were slightly lower in the sham-operated group [range: 58.9 (7.5)–85.4 (11.5)], as compared with the ligated group [range: 80.9 (3.4)–91.4 (3.7); $F(1,23)=18.67$, $P<0.001$]. Overall, these data indicate that there was no evidence for the occurrence of contralateral hyperalgesic or allodynic effects.

3.3. Pharmacological testing

Across all pharmacological tests, mean (S.E.M.) baseline reaction latencies after thermal stimulation of the left, non-operated paw as assessed in the different groups on the day preceding the test day ranged from 17.2 (1.8) to 23.3 (1.8), and, for each compound tested, were not statistically different from each other (outcome of ANOVAs always $P>0.05$). Mean (S.E.M.) baseline reaction latencies of the right paw ranged from 7.8 (0.5) to 12.8 (0.7) across all ligated groups, and ranged from 18.9 (1.9) to 23.3 (1.8) across all sham-operated groups. For each compound tested, ANOVA of the right paw reaction latencies indicated a main group effect (outcome of ANOVAs always $P<0.001$). Post hoc analysis indicated that all ligated groups differed significantly from the respective sham-operated group (always $P<0.001$), but did not differ from each other (always $P>0.05$). Also with respect to mechanical stimulation, baseline withdrawal threshold values

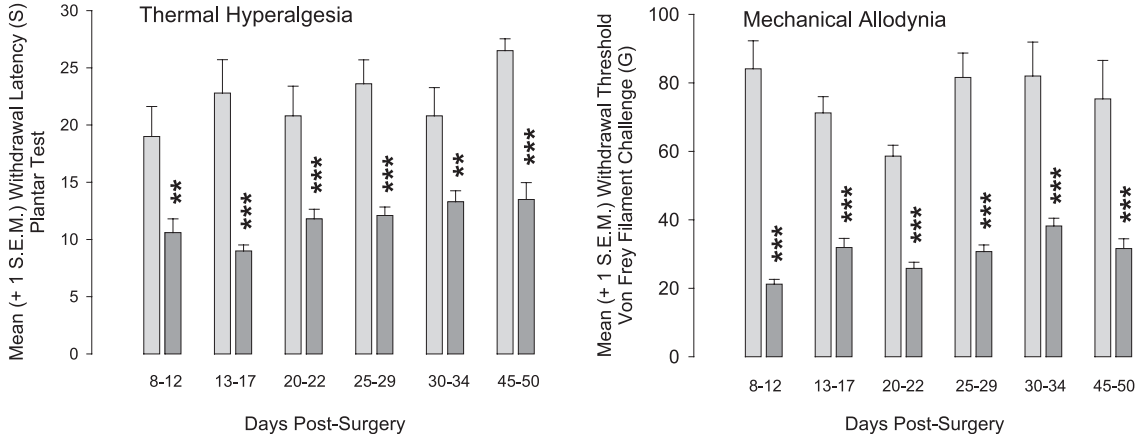

Fig. 1. Time-course of thermal hyperalgesia and mechanical allodynia after unilateral chronic constriction injury of the sciatic nerve (dark grey bars) or sham surgery (light grey bars) in rats. Data were obtained from day 8 to day 55 after surgery, and show the mean (+1 S.E.M.) reaction time latency to thermal stimulation (expressed in seconds), or withdrawal threshold to electronic von Frey filamant challenge (expressed in grams) of the paw of the operated hindlimb. $^{**}P<0.01$, $^{***}P<0.001$ versus sham-operated group ($n=18$, ligated group; $n=7$, sham-operated group).

obtained at the left, non-operated paw, across all pharmacological tests, were not different from each other [outcome of ANOVAs always $P > 0.05$; mean (S.E.M.) values ranged from 65.3 (3.4) to 86.7 (3.1)]. Mean (S.E.M.) baseline withdrawal thresholds after mechanical stimulation of the right paw ranged from 15.6 (1.9) to 38.2 (2.5) across all ligated groups, and ranged from 65.5 (3.1) to 79.0 (1.9) across all sham-operated groups. For each compound tested, ANOVA of the right paw threshold values indicated a main group effect (outcome of ANOVAs always $P < 0.001$). Post hoc analysis indicated that all ligated groups different from the respective sham-operated group (always $P < 0.001$), but did not differ from each other (always $P > 0.05$). Mechanical allodynia and thermal hyperalgesia was reliably present in >90% of the ligated rats.

Morphine (8–32 mg/kg, p.o.) induced a strong attenuation of thermal hyperalgesia and mechanical allodynia; with an ED_{50} value (95% confidence limits) of 12.2 (7.5–19.7) and 15.3 (9.4–24.9) mg/kg, respectively (Fig. 2, outcome of ANOVA in Table 1 for the plantar test and in Table 2 for the von Frey test). Maximal antihyperalgesic

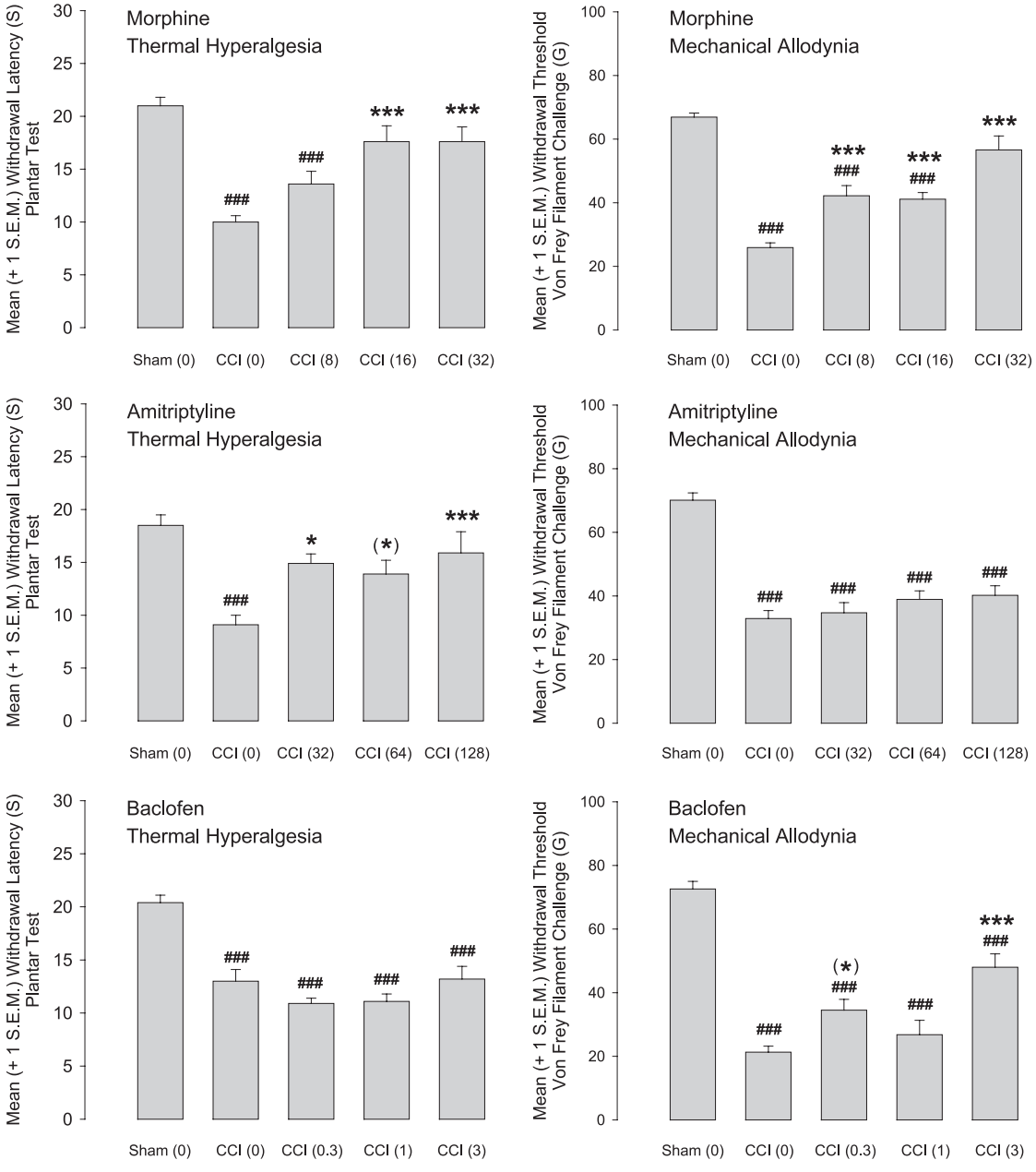

Fig. 2. Effect of morphine (p.o., $t = 60$ min, $n = 12–24$ per group), amitriptyline (p.o., $t = 60$ min, $n = 9–11$ per group), and baclofen (i.p., $t = 30$ min, $n = 11–12$ per group) on thermal hyperalgesia and mechanical allodynia after chronic constriction injury of the sciatic nerve. Doses are expressed in mg/kg and are indicated in brackets. $^{(*)}P = 0.08$, $^{*}P < 0.05$, $^{***}P < 0.001$ versus vehicle-treated ligated group; $^{\#\#\#}P < 0.001$ versus vehicle-treated sham-operated group.

Table 1
Outcome of ANOVAs on reaction latency values in the plantar test

Compound	Left non-operated paw	Right operated paw
Morphine	$F(4,115)=4.64$, $P<0.01$	$F(4,115)=14.04$, $P<0.001$
Amitriptyline	$F(4,45)=6.02$, $P<0.01$	$F(4,45)=7.34$, $P<0.001$
Baclofen	$F(4,51)=0.60$, $P>0.05$	$F(4,51)=21.40$, $P<0.001$
Gabapentin	$F(3,46)=0.97$, $P>0.05$	$F(3,46)=24.57$, $P<0.001$
Carbamazepine	$F(4,48)=6.20$, $P<0.001$	$F(4,48)=26.93$, $P<0.001$
Δ^9-THC[a]	$F(3,68)=0.23$, $P>0.001$	$F(3,68)=55.00$, $P<0.001$
CP 55,940	$F(4,54)=6.20$, $P<0.001$	$F(4,54)=11.09$, $P<0.001$
Rofecoxib	$F(3,44)=5.69$, $P<0.01$	$F(3,44)=21.10$, $P<0.001$
ABT-702	$F(4,55)=2.22$, $P>0.05$	$F(4,55)=23.50$, $P<0.001$
T62	$F(3,50)=0.09$, $P>0.05$	$F(3,50)=76.47$, $P<0.001$
Ketamine	$F(4,55)=2.78$, $P<0.05$	$F(4,55)=58.13$, $P<0.001$
Dizocilpine	$F(2,31)=1.76$, $P>0.05$	$F(2,31)=133.60$, $P<0.001$
Ifenprodil	$F(4,53)=1.11$, $P>0.05$	$F(4,53)=70.24$, $P<0.001$
Ro 25-6981	$F(4,55)=0.13$, $P>0.05$	$F(4,55)=19.97$, $P<0.001$

[a] Δ^9-THC, Δ^9-tetrahydrocannabinol.

efficacy was 69%, obtained at both 16 and 32 mg/kg (Table 3). With respect to the anti-allydonic effect, the MED was 8 mg/kg and the maximal efficacy was 75%, at 32 mg/kg. Morphine less potently also affected reaction latency to thermal stimulation and the withdrawal threshold after mechanical stimulation when the non-operated paw was tested (data not shown; outcome of ANOVA in Tables 1 and 2, respectively). Post hoc analysis indicated that the reaction latency was increased at 32 mg/kg (mean latency of 23.5 s, as compared with 17.7 s in the vehicle-treated ligated group, $P<0.001$). The withdrawal threshold was increased at both 16 and 32 mg/kg (mean threshold of 77.0 g in both groups, as compared with 68.9 g in the vehicle-treated sham-operated group, $P<0.05$). Amitriptyline (32–128 mg/kg, p.o.) induced a strong attenuation of thermal hyperalgesia, with an ED_{50} value of 14.0 (1.1–180.1), but did not affect mechanical allodynia (Fig. 2). Maximal antihyperalgesic efficacy was 72%, at 128 mg/kg. Amitriptyline also affected reaction latency of the non-operated paw, with a significant decrease at 64 mg/kg (mean latency of 12.0 s, as compared with 19.8 s in the vehicle-treated ligated group, $P<0.001$). Baclofen (0.3–3 mg/kg, i.p.) moderately attenuated mechanical allodynia, with an ED_{50} value of about 3 mg/kg, but did not affect thermal hyperalgesia (Fig. 2). Maximal anti-allodynic efficacy was 52%, at 3 mg/kg. Baclofen did not affect either behavioral parameter at the non-operated paw.

Table 2
Outcome of ANOVAs on withdrawal threshold values in the von Frey test

Compound	Left non-operated paw	Right operated paw
Morphine	$F(4,115)=4.38$, $P<0.01$	$F(4,115)=33.33$, $P<0.001$
Amitriptyline	$F(4,45)=0.60$, $P>0.05$	$F(4,45)=33.03$, $P<0.001$
Baclofen	$F(4,51)=0.92$, $P>0.05$	$F(4,51)=37.69$, $P<0.001$
Gabapentin	$F(3,46)=5.07$, $P<0.01$	$F(3,46)=28.38$, $P<0.001$
Carbamazepine	$F(4,48)=1.43$, $P>0.05$	$F(4,48)=56.91$, $P<0.001$
Δ^9-THC[a]	$F(3,68)=11.87$, $P<0.001$	$F(3,68)=79.61$, $P<0.001$
CP 55,940	$F(4,54)=7.25$, $P<0.001$	$F(4,54)=31.58$, $P<0.001$
Rofecoxib	$F(3,44)=0.19$, $P>0.01$	$F(3,44)=108.36$, $P<0.001$
ABT-702	$F(4,55)=2.14$, $P>0.05$	$F(4,55)=40.09$, $P<0.001$
T62	$F(3,50)=0.41$, $P>0.05$	$F(3,50)=46.83$, $P<0.001$
Ketamine	$F(4,55)=3.99$, $P<0.01$	$F(4,55)=99.10$, $P<0.001$
Dizocilpine	$F(2,31)=1.19$, $P>0.05$	$F(2,31)=80.56$, $P<0.001$
Ifenprodil	$F(4,53)=1.77$, $P>0.05$	$F(4,53)=113.12$, $P<0.001$
Ro 25-6981	$F(4,55)=0.74$, $P>0.05$	$F(4,55)=140.65$, $P<0.001$

[a] Δ^9-THC, Δ^9-tetrahydrocannabinol.

Table 3
Overview of maximal antihyperalgesic/anti-allodynic efficacy of the tested compounds

Compound	Thermal hyperalgesia (dose[a])	Mechanical allodynia (dose[a])
Morphine	69% (16–32 mg/kg, p.o.)	75% (32 mg/kg, p.o.)
Amitriptyline	72% (128 mg/kg, p.o.)	20% (128 mg/kg, p.o.)
Baclofen	3% (3 mg/kg, i.p.)	52% (3 mg/kg, i.p.)
Gabapentin	31% (50 mg/kg, i.p.)	35 % (50 mg/kg, i.p.)
Carbamazepine	69% (64 mg/kg, i.p.)	30% (32–64 mg/kg, i.p.)
Δ^9-THC[b]	98% (6 mg/kg, p.o.)	80% (6 mg/kg, p.o.)
CP 55,940	100% (0.05–0.1 mg/kg, i.p.)	47% (0.1 mg/kg, i.p.)
Rofecoxib	45% (30 mg/kg, i.p.)	0% (10–30 mg/kg, i.p.)
ABT-702	23% (3 mg/kg, i.p.)	33% (30 mg/kg, i.p.)
T62	29% (30 mg/kg, i.p.)	10% (30 mg/kg, i.p.)
Ketamine	7% (10 mg/kg, i.p.)	5% (20 mg/kg, i.p.)
Dizocilpine	0%[c] (0.125 mg/kg, i.p.)	0%[c] (0.125 mg/kg, i.p.)
Ifenprodil	25% (20 mg/kg, i.p.)	17% (20 mg/kg, i.p.)
Ro 25-6981	12% (10 mg/kg, i.p.)	8% (20 mg/kg, i.p.)

Efficacy is expressed as percentage reversal of thermal hyperalgesia and mechanical allodynia, respectively.
[a] Dose at which maximal efficacy was obtained.
[b] Δ^9-THC, Δ^9-tetrahydrocannabinol.
[c] Behavioral side-effects precluded testing at 0.25–0.5 mg/kg.

Gabapentin (50–100 mg/kg, i.p.) induced a weak to moderate attenuation of thermal hyperalgesia and mechanical allodynia (Fig. 3). Maximal antihyperalgesic and anti-allodynic efficacy was 31% and 35%, respectively, both obtained at 50 mg/kg. Gabapentin also affected the withdrawal threshold of the non-operated paw, with a significant increase at 100 mg/kg (mean threshold of 85.1 g, as compared with 73.8 g in the vehicle-treated sham-operated group, $P<0.01$). Carbamazepine (16–64 mg/kg, i.p.) induced a strong attenuation of thermal hyperalgesia, with an ED_{50} value of 42.2 (26.2–68.0), and a weak to moderate attenuation of mechanical allodynia (Fig. 3). Maximal antihyperalgesic efficacy was 69%, at 64 mg/kg. The MED for the anti-allydonic effect was 32 mg/kg and the maximal efficacy was 30%, obtained at both 32 and 64 mg/kg. Carbamazepine did not affect reactivity of the non-operated paw, but at the highest dose tested, 3 out of 11 rats could not be tested due to severe behavioral side-effects (i.e., sedation, lying on belly or one side).

Δ^9-Tetrahydrocannabinol (3–6 mg/kg, p.o.) induced a strong attenuation of thermal hyperalgesia and mechanical allodynia; with an ED_{50} value of 3.7 (3.1–4.5) and 4.0 (3.0–5.2) mg/kg, respectively (Fig. 4). Maximal antihyperalgesic efficacy was 98%, at 6 mg/kg. The MED for the anti-allodynic effect was 3 mg/kg and the maximal efficacy was 80%, at 6 mg/kg. Δ^9-Tetrahydrocannabinol less potently also affected the withdrawal threshold of the non-operat-

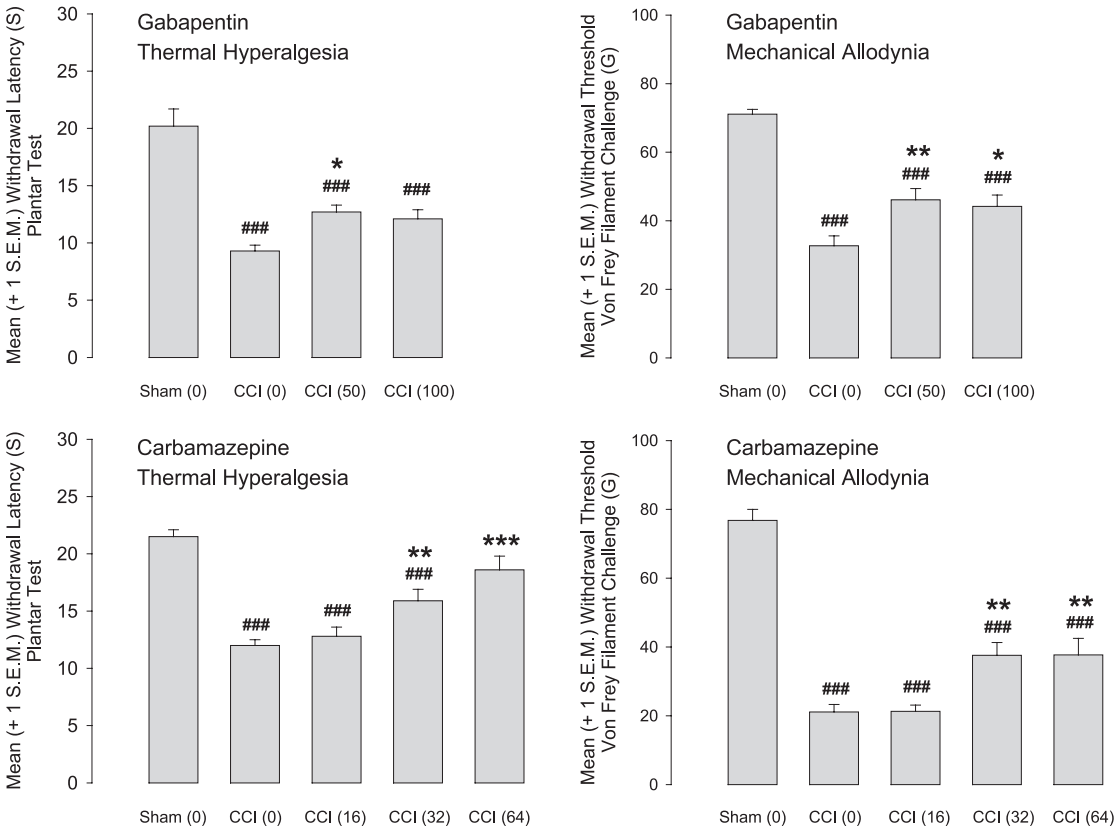

Fig. 3. Effect of gabapentin (i.p., $t=60$ min, $n=11-13$ per group) and carbamazepine (i.p., $t=30$ min, $n=8-12$ per group; at the highest dose 3 out of 11 rats were discarded because behavioral side-effects precluded testing) on thermal hyperalgesia and mechanical allodynia after chronic constriction injury of the sciatic nerve. Doses are expressed in mg/kg and are indicated in brackets. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ versus vehicle-treated ligated group; $^{\#\#\#}P<0.001$ versus vehicle-treated sham-operated group.

ed paw, with a significant increase at 6 mg/kg (mean threshold of 96.5 g, as compared with 79.2 g in the vehicle-treated sham-operated group, $P<0.001$). CP 55,940 (0.025–0.1 mg/kg, i.p.) induced a strong attenuation of thermal hyperalgesia and mechanical allodynia, with an ED_{50} value of <0.25 and about 0.1 mg/kg, respectively (Fig. 4). Antihyperalgesic efficacy was 100%, at both 0.05 and 0.1 mg/kg. The MED for the anti-allodynic effect was 0.05 mg/kg and the maximal efficacy was 47%, at 0.1 mg/kg. CP 55,940 less potently also affected reactivity of the non-operated paw. At the highest dose tested, the reaction latency was increased (mean latency of 24.5 s, as compared with 15.2 s in both the vehicle-treated sham-operated and ligated group, $P<0.001$), as well as the withdrawal threshold (mean threshold of 77.8 g, as compared with 67.3 and 69.9 g, respectively, $P<0.001$).

Rofecoxib (10-30 mg/kg, p.o.) induced a moderate attenuation of thermal hyperalgesia, but did not affect mechanical allodynia (Fig. 5). Maximal efficacy was 45%, at 30 mg/kg. Rofecoxib also affected reaction latency of the non-operated paw, with a significant decrease at both doses (mean latency of 15.1 s in both groups) when compared with the vehicle-treated sham-operated group (mean latency: 20.7 s, $P<0.05$), but not when compared with the vehicle-treated ligated group (mean latency: 14.2 s).

ABT-702 (3–30 mg/kg, i.p.) induced a relatively weak attenuation of mechanical allodynia, but did not affect thermal hyperalgesia (Fig. 6). Maximal efficacy was 33%, at 30 mg/kg. ABT 702 did not affect either behavioral parameter when the non-operated paw was tested. T62 (15–30 mg/kg, i.p.) induced a weak attenuation of thermal hyperalgesia, but did not affect mechanical allodynia (Fig. 6). Maximal efficacy was 29%, at 30 mg/kg. The non-operated paw was not affected by T62.

Ketamine (5–20 mg/kg, i.p.) had no significant antihyperalgesic or anti-allodynic effect (Fig. 7). Although ANOVA revealed a main group effect on reaction latency of the non-operated paw, post hoc analysis indicated that none of the drug-treated groups differed from the vehicle-treated groups. With respect to the withdrawal threshold of the non-operated paw, a significant increase was obtained at 5 mg/kg (mean value of 81.6 g) when compared with the vehicle-treated non-operated group (mean value of 68.6 g, $P<0.05$). Dizocilpine (0.125 mg/kg, i.p.) did not affect any behavioral parameter (Fig. 7). Side-effects (i.e., ataxia, stereotypies, tumbling) precluded testing of higher doses.

Ifenprodil (5–20 mg/kg, i.p.) induced a weak attenuation of thermal hyperalgesia and mechanical allodynia (Fig. 8). Maximal antihyperalgesic and anti-allodynic efficacy was 25% and 17%, respectively, at 20 mg/kg. The non-operated

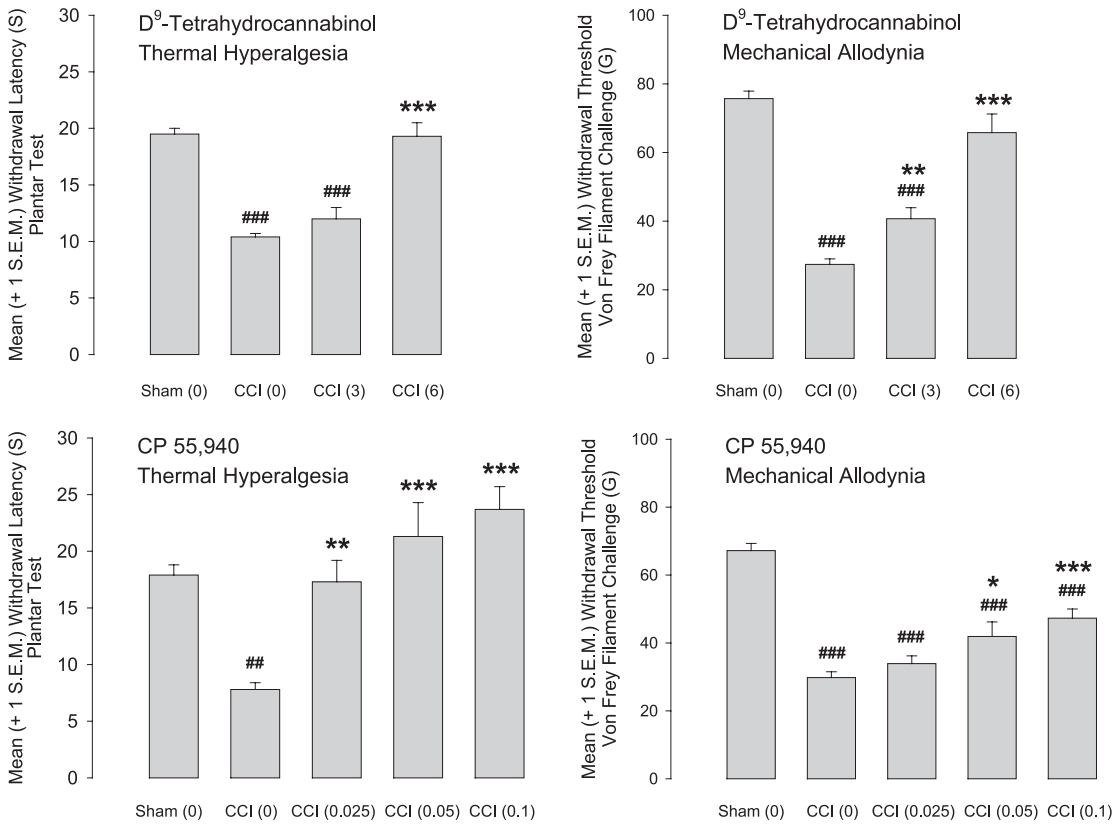

Fig. 4. Effect of the cannabinoids Δ^9-tetrahydrocannabinol (p.o., $t=60$ min, $n=12-24$ per group) and CP 55,940 (i.p., $t=30$ min, $n=11-13$ per group) on thermal hyperalgesia and mechanical allodynia after chronic constriction injury of the sciatic nerve. Doses are expressed in mg/kg and are indicated in brackets. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ versus vehicle-treated ligated group; $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ versus vehicle-treated sham-operated group.

paw was not affected by ifenprodil. Ro 25-6981 (5–20 mg/kg, i.p.) did not affect any behavioral parameter (Fig. 8).

4. Discussion

The present study assessed the pharmacological sensitivity of the chronic constriction injury model of neuropathic pain under standardized experimental conditions. Thermal hyperalgesia and mechanical allodynia developed within one week post-surgery and were reliably present for at least 7 weeks. Both symptoms were attenuated to variable degrees by a number of compounds used clinically for the symptomatic treatment of neuropathic pain, as well as by some experimental compounds previously reported to be effective in animal models of neuropathic pain.

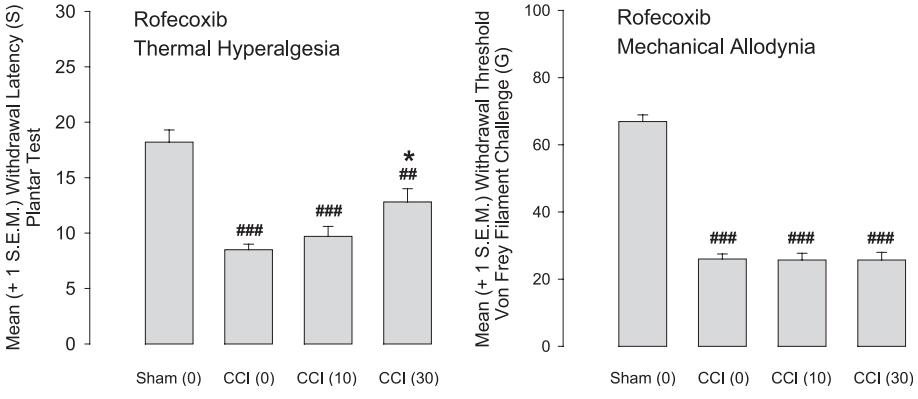

Fig. 5. Effect of the cyclooxygenase-2 inhibitor rofecoxib (i.p., $t=60$ min, $n=12$ per group) on thermal hyperalgesia and mechanical allodynia after chronic constriction injury of the sciatic nerve. Doses are expressed in mg/kg and are indicated in brackets. $^{*}P<0.05$ versus vehicle-treated ligated group; $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ versus vehicle-treated sham-operated group.

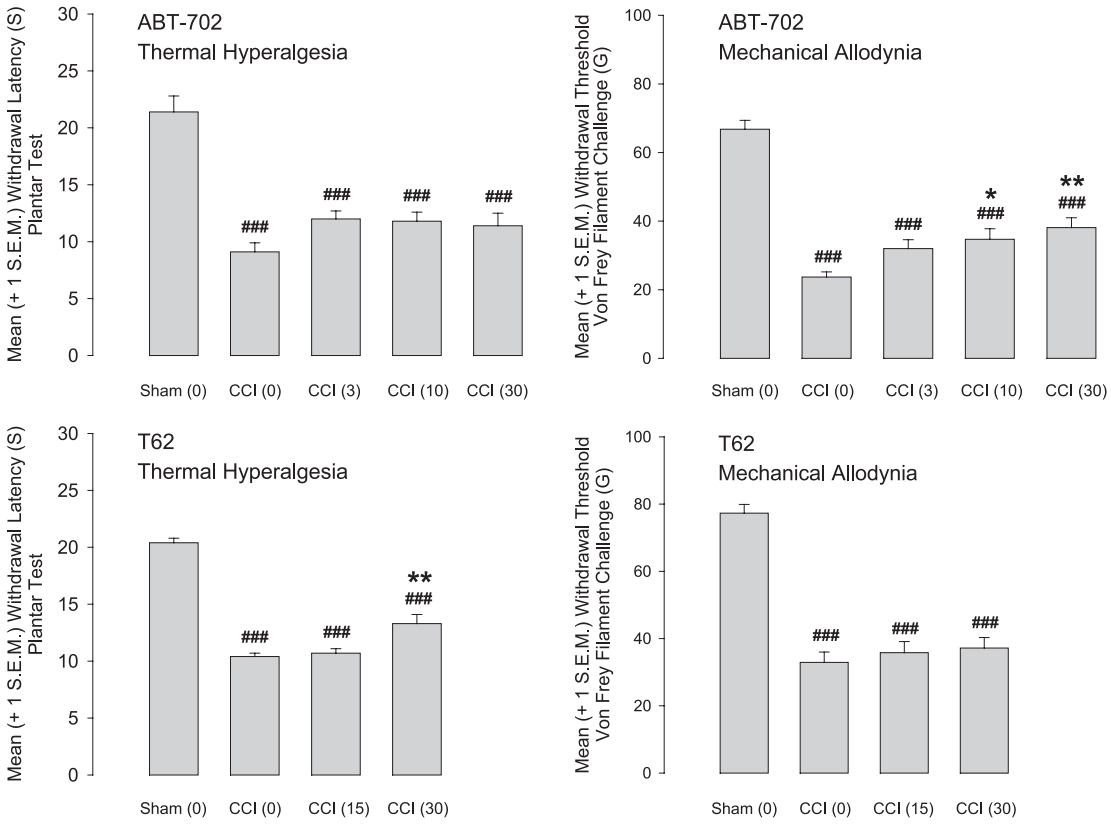

Fig. 6. Effect of the adenosine kinase inhibitor ABT-702 (i.p., $t = 30$ min, $n = 12$ per group) and the adenosine A_1 receptor positive allosteric modulator T62 (i.p., $t = 90$ min, $n = 13-14$ per group) on thermal hyperalgesia and mechanical allodynia after chronic constriction injury of the sciatic nerve. Doses are expressed in mg/kg and are indicated in brackets. $^{*}P < 0.05$, $^{**}P < 0.01$ versus vehicle-treated ligated group; $^{\#\#\#}P < 0.001$ versus vehicle-treated sham-operated group.

The present study further validates the chronic constriction injury model for the study of neuropathic pain and supports its use for the identification of novel treatments.

The finding that thermal hyperalgesia and mechanical allodynia were reliably present for at least 7 weeks post-surgery is consistent with the original report by Bennett and Xie (1988). When both symptoms were assessed in the tibial nerve injury model of neuropathic pain using the same methodology as in the present study, it was found that mechanical allodynia was more reliably present than thermal hyperalgesia (Hofmann et al., 2003). This supports the notion that the pathophysiological processes underlying the development and maintenance of thermal hyperalgesia after nerve ligation and disection are, at least partially, different (for discussion, see Bridges et al., 2001). In addition, it was found that ligation induced no contralateral effects, as evidenced by similar reaction latency and withdrawal threshold values upon thermal and mechanical stimulation of the non-operated paw. This was also reported in the original description of the model (Bennett and Xie, 1988), and contrasts with the presence of contralateral effects in the spared nerve injury model (Erichsen and Blackburn-Munro, 2002; but see Decosterd and Woolf, 2000), the partial sciatic nerve injury model (Seltzer et al., 1990), and the spinal nerve ligation model (Kim and Chung, 1992).

Acute oral administration of the opioid morphine almost completely reversed thermal hyperalgesia and mechanical allodynia. Antihyperalgesic and anti-allodynic properties were also reported in the same model when the compound was administered by other routes of administration (e.g., Attal et al., 1991; Koch et al., 1996; Pelissier et al., 2003). Although it is still controversial whether opiates are clinically effective against neuropathic pain (for discussion, see Dellemijn, 1999), it should be noted that the antihyperalgesic and anti-allodynic efficacy of morphine, as obtained in the present study, showed some degree of specificity. Indeed, for both symptoms it could be demonstrated that the compound more potently (about twofold) affected behavioral sensitivity of the ligated paw, than that of the non-operated paw. This suggests that the antihyperalgesic and anti-allodynic properties of morphine were not confounded by antinociceptive or anaesthetic properties (or behavioral side-effects).

Although the tricyclic antidepressant amitrityline and the muscle relaxant baclofen were both active in the model, their profile of activity was different from each other and from that of morphine. Thus, whereas amitriptyline was only effective against thermal hyperalgesia, baclofen was found to be only active against mechanical allodynia. Smith et al. (1994) also reported efficacy of baclofen against

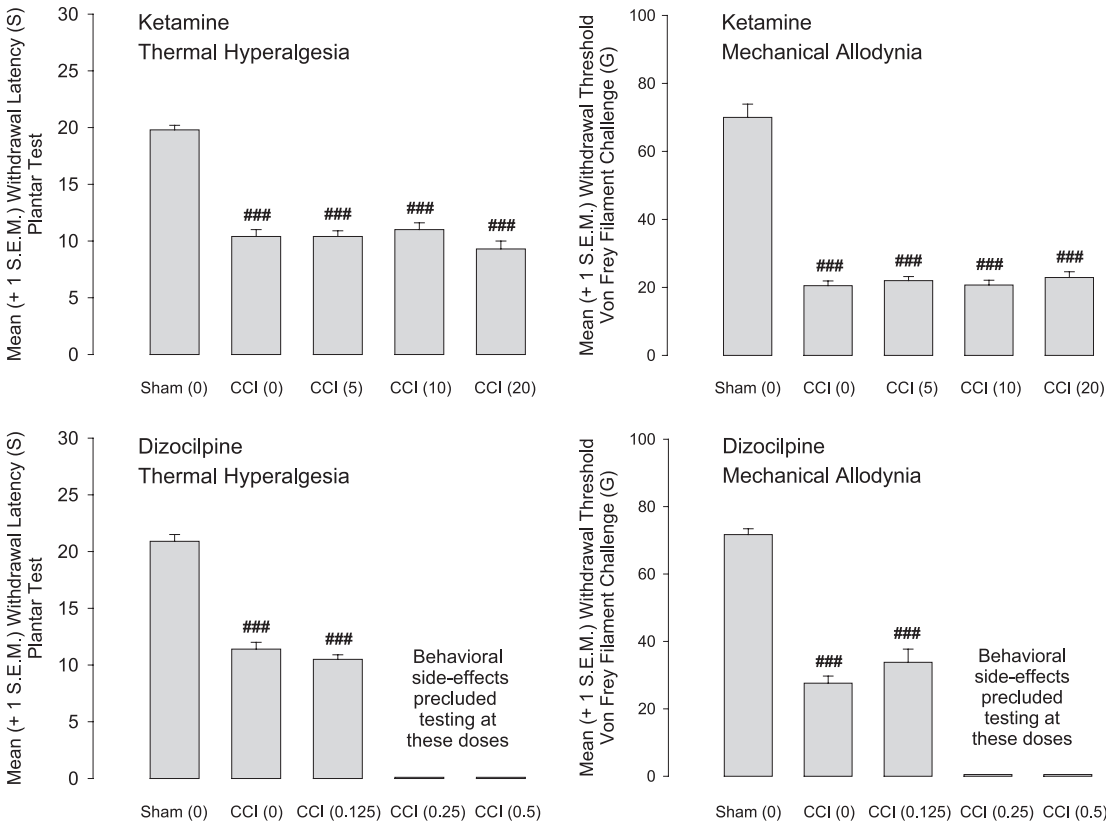

Fig. 7. Effect of ketamine (i.p., $t = 30$ min, $n = 12$ per group) and dizocilpine (i.p., $t = 30$ min, $n = 11-12$ per group; at the two highest doses behavioral side-effects precluded testing) on thermal hyperalgesia and mechanical allodynia after chronic constriction injury of the sciatic nerve. Doses are expressed in mg/kg and are indicated in brackets. $^{###}P < 0.001$ versus vehicle-treated sham-operated group.

mechanical allodynia in the same model, whereas amitriptyline was found to be effective against mechanical hyperalgesia (Koch et al., 1996). Efficacy of these compounds against chronic pain symptoms was relatively specific, as reactivity of the non-operated paw was less potently (twofold for amitriptyline, see also Koch et al., 1996; at least twofold for baclofen) affected than reactivity of the ligated paw.

The anticonvulsants gabapentin and carbamazepine induced a relatively similar profile of activity, as they both showed significant antihyperalgesic and anti-allodynic properties in the same dose range. Gabapentin was previously reported to be effective in this model, although, as in the present study, the magnitude of the effect appeared to be relatively weak, with a relatively small therapeutic window (sedation occurs at twice the MED; Boyce et al., 1999; Hunter et al., 1997; Xiao and Bennett, 1996). Also for carbamazepine, antihyperalgesic properties were reported in this model (Koch et al., 1996), but, again, the therapeutic window appeared to be relatively small (severe behavioral side-effects confounded testing at the highest dose).

The dissociative anaesthetics and nonselective *N*-methyl-D-aspartate receptor antagonists ketamine and dizocilpine failed to show efficacy in the tested dose range. Although Pelissier et al. (2003) reported an antihyperalgesic effect for ketamine in this model, its magnitude was relatively weak and only obtained at high doses which coincide with ataxia and stereotyped behavior (>50 mg/kg, s.c.). In the case of dizocilpine, these behavioral side-effects precluded testing of doses higher than 0.125 mg/kg, i.p. Although dizocilpine was reported to be effective in the chronic constriction injury model at a dose devoid of behavioral side-effects, antihyperalgesic properties only became apparent after repeated administration (Begon et al., 2000). Boyce et al. (1999) were able to demonstrate efficacy of this compound against mechanical allodynia, but this occurred only at doses which induced a performance dificit in a rotarod device. Erichsen and Blackburn-Munro (2002) also reported a lack of activity of dizocilpine in the spared nerve injury model. Together, these findings suggest that the clinical utility of nonselective *N*-methyl-D-aspartate receptor antagonists for the treatment of neuropathic pain is compromised by their (very) small therapeutic window. Although it was argued that *N*-methyl-D-aspartate receptor NR2B-selective antagonists, such as ifenprodil (Avenet et al., 1997) and Ro 25-6981 (Mutel et al., 1998) would be able to overcome the drawback of a small therapeutic window (for discussion, see Boyce et al., 1999), the present study failed to demonstrate clear efficacy of these compounds. This finding contrasts with the study of Boyce et al. (1999), in which both compounds were reported to have pronounced efficacy against mechanical allodynia in the chronic constriction injury model. The present lack of

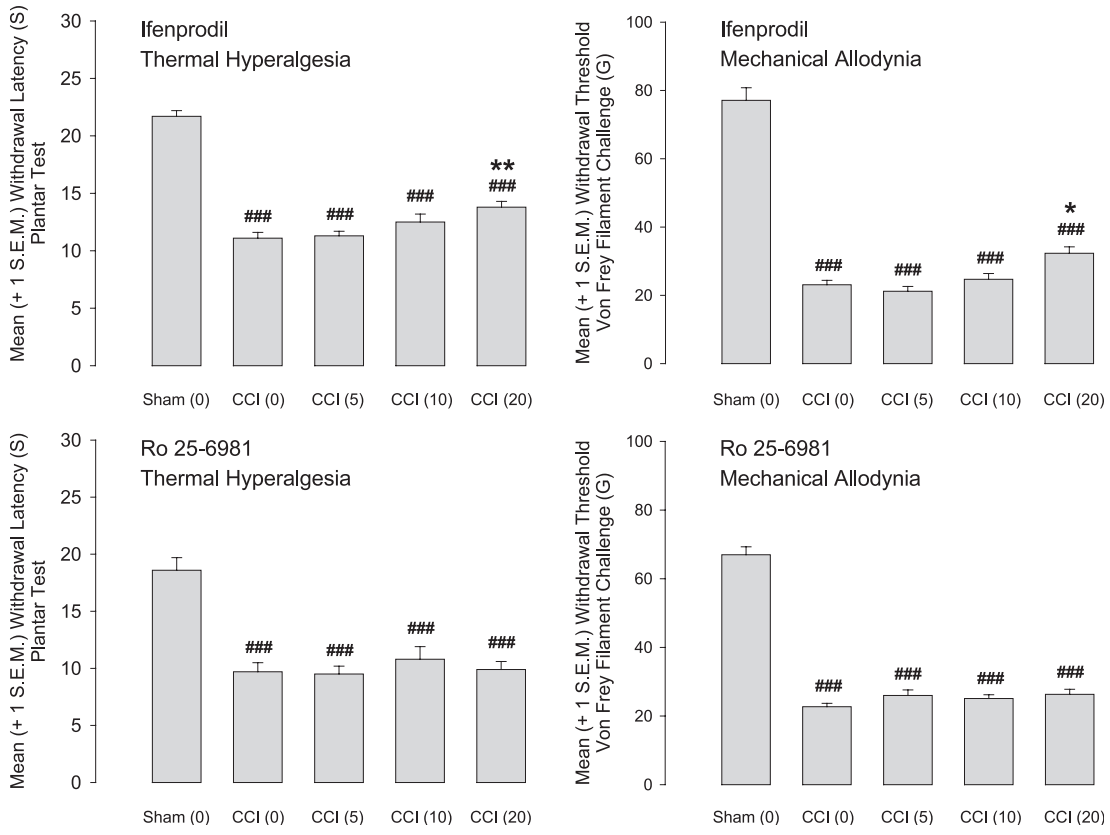

Fig. 8. Effect of the *N*-methyl-D-aspartate receptor NR2B-selective antagonists ifenprodil (i.p., $t = 30$ min, $n = 11-12$ per group) and Ro 25-6981 (i.p., $t = 30$ min, $n = 12$ per group) on thermal hyperalgesia and mechanical allodynia after chronic constriction injury of the sciatic nerve. Doses are expressed in mg/kg and are indicated in brackets. $^{*}P < 0.05$, $^{**}P < 0.01$ versus vehicle-treated ligated group; $^{###}P < 0.001$ versus vehicle-treated sham-operated group.

efficacy can most likely not be ascribed to inappropriate dose selection as the same dose range and route of application was used as in the Boyce et al. (1999) study, and behavioral activity was demonstrated in an operant behavior assay under exactly the same application conditions (De Vry and Jentzsch, 2003). It therefore remains unclear whether *N*-methyl-D-aspartate receptor NR2B-selective antagonists are valuable treatment alternatives.

Somewhat surprisingly, the anti-inflammatory cyclooxygenase-2 inhibitor rofecoxib (Chan et al., 1999) was found to have moderate activity against thermal hyperalgesia. Although anti-inflammatory agents have not been extensively studied in animal models of neuropathic pain (Martin and Eisenach, 2001), it is generally assumed that they are not effective against neuropathic pain. Therefore, the antihyperalgesic effect may indicate that inflammatory processes contribute to the pathophysiology of the model (possibly due to the presence of ligation material, Lindenlaub and Sommer, 2000).

Of particular interest is the finding that the cannabinoids Δ^9-tetrahydrocannabinol and CP 55,940 showed pronounced antihyperalgesic and anti-allodynic properties. Other cannabinoids, such as (*R*)-4,5-dihydro-2-methyl-4(4-morpholinylmethyl)-1-(1-naphthalenylcarbonyl)-6*H*-pyrrolo[3,2,1-ij] quinolin-6-one (WIN 55,212-2) and 3-[2-cyano-3-(trifluoromethyl)phenoxy]phenyl-4,4,4-trifluoro-1-butanesulfonate (BAY 59-3074), were also reported to be effective in this model (De Vry et al., in press; Herzberg et al., 1997). The antihyperalgesic and anti-allodynic properties of these compounds appear to be relatively specific, and can clearly be dissociated from their antinociceptive properties (for discussion, see De Vry et al., 2004a, in press; Siegling et al., 2001). Together, these findings indicate that cannabinoids may offer new opportunities for the treatment of neuropathic pain (for review, see Pertwee, 2001).

Finally, both the adenosine kinase inhibitor ABT-702 (Kowaluk et al., 2000) and the adenosine A_1 receptor positive allosteric modulator T62 (Li et al., 2002) were found to have relatively weak efficacy against mechanical allodynia and thermal hyperalgesia, respectively. As assessed in the spinal nerve ligation model, both compounds were previously reported to have some efficacy against mechanical allodynia (Kowaluk et al., 2000; Li et al., 2002). However, due to their limited efficacy and their relatively small therapeutic window (in the present study effective doses of both compounds also induced sedation and flat body posture), such compounds do not appear to have advantages above currently available treatment.

In conclusion, the present study offered a relatively broad pharmacological validation of the chronic constriction injury

model of neuropathic pain. Further clinical research is needed to clarify whether the differential profile of activity of these compounds in the model is reflected by differential efficacy against various clinical symptoms of neuropathic pain.

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